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(54) VARIANT NUCLEOSIDE-5'-PHOSPHATE-PRODUCTIVE ENZYME

(57)Abstract

PROBLEM TO BE SOLVED: To provide a new variant nucleoside—5'—phosphate—productive enzyme improved in nucleoside—5'—phosphate productivity, to provide a new means for obtaining the enzyme, and to provide uses of the above enzyme.

SOLUTION: This variant nucleoside-5'-phosphate-productive enzyme has such characteristics that there are one Lys residue, two Arg residues and two His residues, the $C\alpha$ - $C\alpha$ distances therebetween stand within a specific range, respectively, there is a space to which a nucleoside is bound in the proximity thereto, there have phosphate group transfer activity and/or phosphatase activity, and thereby nucleoside-5'-phosphate productivity is improved. A method for producing this enzyme by determining a variation based on the X-ray crystal structural analysis of the corresponding well-known enzyme is provided. By this method, the objective enzyme of higher activity can be easily obtained.

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CLAIMS

[Claim(s)]

[Claim 1]In a nucleoside 5'-phosphoric acid production enzyme, one Lys residue, two Arg residue, Two His residue exists and there is distance between such Calpha within limits shown in drawing 1, And a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity of an enzyme which has a space which a nucleoside combines near the, and which has phosphoryl-group-transfer activity and/or phosphatase activity improved.

[Claim 2] The variant nucleoside 5'-phosphoric acid production enzyme according to claim 1 which is that to which the origin of an enzyme belongs to the Escherichia bacteria, the Morganella bacteria, Providencia bacteria, the Enterobacter bacteria, KUREBUJIERA group bacteria, or the Enterobacter bacteria.

[Claim 3]It carries out based on structure coordinates shown in atomic coordinate data produced by conducting X ray crystal structure analysis of the crystal of Escherichia BURATTAE origin acid phosphatase, The variant nucleoside 5'-phosphoric acid production enzyme according to claim 1 which presumed a bond form with nucleosides, such as inosine and guanosine, and a phosphoric acid compound of those and whose nucleoside 5'-phosphoric acid productivity improved by substitution, such as amino acid residue and/or a supplementary factor, an addition, and deletion.

[Claim 4]A following position of an amino acid sequence of Escherichia BURATTAE origin acid

phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A): 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.

[Claim 5]With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, when amino acid sequence alignment with Escherichia BURATTAE origin acid phosphatase is carried out, A following position of an amino acid sequence of Escherichia BURATTAE origin acid phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A): 16, 67–76, 78–79, 96, 99–100, 102–104, 106–108, 115 and 140, 148–154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position corresponding to 157, 179, and 183.

[Claim 6]With an enzyme which has phosphoryl—group—transfer activity and/or phosphatase activity, when alignment with the three—dimensional structure of Escherichia BURATTAE origin acid phosphatase is performed by the TOREDDINGU method, A following position of an amino acid sequence of Escherichia BURATTAE origin acid phosphatase (residue in Ser72 of Escherichia BURATTAE acid phosphatase, or Ser72 to 10A): 16, 67–76, 78–79, 96, 99–100, 102–104, 106–108, 115 and 140, 148–154, a variant nucleoside 5'–phosphoric acid production enzyme that change has produced in at least one position corresponding to 157, 179, and 183.

[Claim 7] The origin of an enzyme is of the Enterobacter aerogenes origin, and in the amino acid sequence The 14th leucine residue, The 61st leucine residue, the 63rd alanine residue, the 64th glutamate residue, The 67th asparagine residue, the 69th serine residue, the 70th alanine residue, The 71st glycine residue, the 72nd glycine residue, the 101st isoleucine residue, The 102nd glutamate residue, the 133rd threonine residue, the 134th glutamate residue, A variant nucleoside 5'-phosphoric acid production enzyme in which at least one amino acid residue is replaced by other amino acid

residue among the 138th leucine residue, the 149th threonine residue, and the 151st isoleucine residue.

[Claim 8]. Became final and conclusive from an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or a spacial configuration produced by conducting X ray crystal structure analysis of the crystal of a complex of it and molybdic acid. A manufacturing method of a variant nucleoside 5'-phosphoric acid production enzyme manufacturing an enzyme variant whose nucleoside 5'-phosphoric acid productivity improved an active site of this enzyme, and/or amino acid residue which is in less than 10A from it by carrying out substitution, an addition, and deletion. [Claim 9]How to use structure coordinates of Escherichia BURATTAE origin acid phosphatase, and to manufacture inhibitor of phosphatase or phosphotransferase.

[Claim 10]One crystal of the complexes of an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or it and molybdic acid.

[Claim 11]A crystal of Escherichia BURATTAE origin acid phosphatase which has space group P6 $_3$ 22 of a hexagonal system.

[Claim 12]A crystal of Escherichia BURATTAE origin acid phosphatase G74 D/I153T enzyme variant which has space group P2 ₁2 ₁2 ₁ of an ortho rhombic system.

[Claim 13]A crystal of a complex (reaction intermediate analog) of Escherichia BURATTAE origin acid phosphatase and molybdic acid which have space group P3 121 of a trigonal system.

[Claim 14]A gene which encodes an enzyme of a statement in any 1 paragraph of claims 1-7.

[Claim 15]A recombinant DNA containing the gene according to claim 14.

[Claim 16] A microorganism which holds the recombinant DNA according to claim 15.

[Claim 17]An enzyme given in any 1 paragraph of claims 1-7, or a microorganism containing it, Or a manufacturing method of nucleoside 5'-phosphoric acid making the microorganism according to claim 16 act on a nucleoside and a phosphate donor, making nucleoside 5'-phosphoric acid generate, and extracting this.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention] [0001]

[Field of the Invention] This invention relates to a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity improved, and a manufacturing method for the same. This invention relates to enzymes useful to manufacture of the above-mentioned enzyme. This invention relates to the microorganism which holds the recombinant DNA containing the gene which encodes the useful aforementioned enzyme variant in the manufacturing method, and this gene, and this recombinant DNA, concerning the manufacturing method of nucleoside 5'-phosphoric acid. Nucleoside 5'-phosphoric acid is useful as a seasoning, medicines, and those raw materials. It is based on this invention having succeeded in the break through of the proteinic new spacial configuration by X ray crystal-structure-analysis art, and this spacial configuration has possibilities which are not restricted to a microorganism.

[0002]

[Description of the Prior Art] As a method of phosphorylating a nucleoside biochemically and manufacturing nucleoside 5'-phosphoric acid cheaply and efficiently, By making a specific microbial cell act on the phosphate donor chosen from the group which comprises a nucleoside and polyphosphoric acid (salt), phenylphosphoric acid (salt), and carbamyl phosphate under acid conditions. The method of generating nucleoside 5'-phosphoric acid efficiently is developed, without being accompanied by the byproduction of nucleoside 2'-phosphoric acid and a nucleoside 3'phosphoric acid isomer (JP,7-231793,A). Then, the gene which encodes acid phosphatase is acquired from Escherichia BURATTAE (Escherichia blattae) and Morganella MORUGANI (Morganella morganii), It was checked by carrying out the extensive manifestation of this gene by Escherichia coli in gene engineering that the productivity of nucleoside 5'-phosphoric acid improves further. The structure of this acid phosphatase is shown in drawing 2. Drawing 2 Namely, Escherichia BURATTAE origin acid phosphatase. The amino acid sequence of (writing it as EB-AP hereafter) Morganella MORUGANI. Salmonella typhimurium (Salmonella typhimurium), They are an amino acid sequence of ZAIMO monas MOBIRISU (Zymomonas mobilis) origin acid phosphatase, and the aligned figure. The base sequence of the gene of each acid phosphatase and the amino acid sequence of the enzyme by which a code is carried out are shown in the array numbers 1-8 of an array table. An asterisk shows the saved residue by drawing 2. After aligning the field of secondary structure, it was shown with a stick. The portion enclosed with the line of the rectangular head shows the motif which is common between acid phosphatase families. The motif consists of three domains, the 1KXXXXXXRP array number 121, the 2PSGH array number 122, and 3SRXXXXXHXXXD array number 123**. Here, X is arbitrary amino acid. This acid phosphatase (drawing 2) has the superior phosphatase activity which decomposes nucleoside 5'-phosphoric acid into a nucleoside in a wild type although it has phosphoryl-grouptransfer activity, and there was a fault into which the accumulated nucleoside 5'-phosphoric acid will be decomposed. Then, generate many enzyme variants at random and the variant acid phosphatase whose phosphoryl-group-transfer activity improved relatively as compared with phosphatase activity from the inside is found out. It was shown by by carrying out the extensive manifestation of this variant acid phosphatase gene that the productivity of nucleoside 5'-phosphoric acid improves by leaps and bounds (JP,9-37785,A). The compatibility of this variant phosphatase over a nucleoside is

improving.

It is thought that phosphoryl—group—transfer activity has been improved by that cause. The above—mentioned Escherichia BURATTAE origin variant acid phosphatase (= G74 D/I153T enzyme variant), Although phosphoryl—group—transfer activity is weaker than G72 D/I151T enzyme variant in which Morganella MORUGANI origin acid phosphatase (MM-AP) corresponds, The 10 residue substitution L63 Q/A65 Q/E66 A/N69 D/S71 A/S72 A/G74 D/T135 K/E136 D/I153T enzyme variant which replaced eight amino acid residue by the amino acid of MM-AP corresponding on the primary structure (henceforth) It was only shown that 10 residue substitution variant EB-AP and description acquire phosphoryl—group—transfer activity almost equivalent to G72 D/I151T variant MM-AP (JP,10-201481,A).

[0003]

[Problem(s) to be Solved by the Invention] The G74 D/I153T variant enzyme gene of the Escherichia BURATTAE origin acid phosphatase (EB-AP) whose above-mentioned productivity improved, the method of producing nucleoside 5'-phosphoric acid is established by carrying out the extensive manifestation of the 10 residue substitution variant enzyme gene by Escherichia coli — having had (JP,9-37785,A, JP,10-201481,A) — variant EB-AP whose productivity improved is desired. This invention makes it a technical problem to aim at further improvement in nucleoside 5'-phosphoric acid productivity by designing variant EB-AP based on the three-dimensional structure of EB-AP. [0004]

[Means for Solving the Problem] If this invention is outlined, it will be as enumerating below. In a nucleoside 5'-phosphoric acid production enzyme, (1) One Lys residue, Two Arg residue and two His residue exist, and there is distance between such Calpha within limits shown in district And a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity of an enzyme which has a space which a nucleoside combines near the, and which has phosphoryl-group-transfer activity and/or phosphatase activity improved.

- (2) A variant nucleoside 5'-phosphoric acid production enzyme given in (1) paragraph which is that to which the origin of an enzyme belongs to the Escherichia bacteria, the Morganella bacteria, Providencia bacteria, the Enterobacter bacteria, KUREBUJIERA group bacteria, or the Enterobacter bacteria.
- (3) It carries out based on structure coordinates shown in atomic coordinate data produced by conducting X ray crystal structure analysis of the crystal of EB-AP, A variant nucleoside 5'-phosphoric acid production enzyme given in (1) paragraph given nucleoside 5'-phosphoric acid productivity improved by presuming a bond form with nucleosides, such as inosine and guanosine, and carrying out substitution, such as amino acid residue and/or a supplementary factor, an addition, and deletion.
- (4). A following position of an amino acid sequence of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A: 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.
- (5) With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity. When amino acid sequence alignment with EB-AP is carried out, A following position of amino acid sequence ** of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A: 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.

 (6) With an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity. When alignment with the three-dimensional structure of EB-AP is performed by the TOREDDINGU method, A following position of amino acid sequence ** of EB-AP. amino acid Ser72 of EB-AP. Or residue in Ser72 to 10A: 16, 67-76, 78-79, 96, 99-100, 102-104, 106-108, 115 and 140, 148-154, a variant nucleoside 5'-phosphoric acid production enzyme that change has produced in at least one position of 157, 179, and 183.

[0005](7) The origin of an enzyme is of the Enterobacter aerogenes origin, In the amino acid sequence, the 14th leucine residue, the 61st leucine residue, The 63rd alanine residue, the 64th glutamate residue, the 67th asparagine residue, The 69th serine residue, the 70th alanine residue, the 71st glycine residue, The 72nd glycine residue, the 101st isoleucine residue, the 102nd glutamate

residue, The 133rd threonine residue, the 134th glutamate residue, the 138th leucine residue, A variant nucleoside 5'-phosphoric acid production enzyme in which at least one amino acid residue is replaced by other amino acid residue among the 149th threonine residue and the 151st isoleucine residue.

- (8) An enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, . Or became final and conclusive from a spacial configuration produced by conducting X ray crystal structure analysis of the crystal of a complex of it and molybdic acid. amino acid residue (constituted by Lys1 ** and two Arg(s) His two) located in an active site of this enzyme and/or and amino acid residue in less than 10A by carrying out substitution, an addition, and deletion, A manufacturing method of a variant nucleoside 5'-phosphoric acid production enzyme manufacturing an enzyme variant whose nucleoside 5'-phosphoric acid productivity improved.
- (9) How to use structure coordinates of Escherichia BURATTAE origin acid phosphatase, and to manufacture inhibitor of phosphatase or phosphotransferase.
- [0006](10) One crystal of the complexes of an enzyme which has phosphoryl-group-transfer activity and/or phosphatase activity, or it and molybdic acid.
- (11) A crystal of EB-AP which has space group P6 322 of a hexagonal system.
- (12) A crystal of EB-AP G74 D/I153T enzyme variant which has space group P2 $_1^2$ $_1^2$ of an orthorhombic system.
- (13) A crystal of a complex (reaction intermediate analog) of EB-AP and molybdic acid which have space group P3 ₁21 of a trigonal system.

[0007](14) A gene which encodes an enzyme of a statement in any 1 paragraph of (1) - (7) paragraph.

- (15) A recombinant DNA which contains a gene of a statement in (14) paragraphs.
- (16) A microorganism which holds a recombinant DNA of a statement in (15) paragraphs.

[0008](17) An enzyme given in any 1 paragraph of (1) - (7) paragraph, or a microorganism containing it, Or a manufacturing method of nucleoside 5'-phosphoric acid making a microorganism given in (16) paragraphs act on a nucleoside and a phosphate donor, making nucleoside 5'-phosphoric acid generate, and extracting this.

[0009]Based on the three-dimensional structure of EB-AP, this invention builds a bond form model with a nucleoside, and provides a production method of nucleoside 5'-phosphoric acid using variant EB-AP designed based on it.

[0010]

[Embodiment of the Invention] Hereafter, this invention is explained concretely.

- (1) In order to determine the proteinic three-dimensional structure by X ray crystal structure analysis, it is necessary to crystallize protein (details were shown in Example 1-3). In order to crystallize protein, pH, the kind of buffer, the concentration of a buffer and the kind of precipitant, the concentration of a precipitant, the concentration of additive agents, such as metal, the concentration of protein, proteinic purity, etc. must determine many parameters by trial and error. Therefore, before obtaining a crystal, usually the time for several months several years will be taken, and there is also a case where a crystal is not obtained against a great labor, plentifully, although crystallization is indispensable for three-dimensional structure determination other than this being also alike there are a purification method of a proteinic high grade, stable Conservation Act whose protease resistance it is high-density and is strong, and also usefulness industrial as a process before fixed use of an enzyme.
- (2) Irradiate the produced crystal with X-rays and collect diffraction data. A crystal protein has plentifully a case where X-ray irradiation receives a damage and diffraction ability deteriorates. In that case, a crystal is rapidly cooled at about -173 **, and the low temperature thermometry which collects diffraction data in the state has been spreading recently. in addition a crystal does not collapse when cooling a system it is necessary to devise solvent composition so that the whole may become vitrified
- (3) In addition to diffraction data, topology is needed in order to conduct crystal structure analysis. Since the spacial configuration of EB-AP of the protein of a relative is strange, a phase problem must be solved by a heavy atom isomorphous replacement method. A heavy atom isomorphous

replacement method is a method of introducing a metal atom with big atomic numbers, such as mercury and platinum, into a crystal, and acquiring topology using contribution of X diffraction DETAHE of the big X-ray scattering ability of a metal atom. If the spacial configuration of wild type EB-AP is determined, the crystal structure of analogs, such as an enzyme variant and a reaction intermediate analog, can be determined with the molecular replacement method which used it. A molecular replacement method is the technique of performing structure determination in the protein which wants to determine a crystal structure using the spacial configuration, when the spacial configuration of the protein of a relative is known. For example, if the spacial configuration of the wild type of a certain protein is known, a molecular replacement method is applicable to the crystal structure determination of the variant protein or the protein by which chemical modification was carried out. About G74 D/I153T variant EB-AP, a crystal structure is determined for a molecular mechanism break through of the improvement in nucleoside compatibility by two amino acid substitution. About a reaction intermediate analog, in order to build a bond form model with a nucleoside, a crystal structure is determined. A nucleoside is changed into nucleoside 5'-phosphoric acid after the phosphate group supplied combines with EB-AP in the state where the covalent bond was carried out, i.e., reaction intermediate. Since the reaction intermediate of EB-AP was unstable, the structure could not be caught, but since it was not hydrolyzed instead of phosphoric acid when molybdic acid was the reaction intermediate analog which carried out the covalent bond, I thought that structure determination was possible. Details were shown in Examples 4, 6, and 7. [0011](4) On computer graphics (CG), based on the molybdic acid connecting position in the threedimensional structure of a reaction intermediate analog, make a nucleoside fit the hollow of the neighborhood and build a bond form model (drawing 3). A program like QUANTA of MSI (United States) and INSIGHT II is used for construction of a model, for example. Drawing 3 is a photograph which shows the crystal structure of the above-mentioned bond form model. Details were shown in Examples 5 and 8.

(5) Observe coupled models well and design the variation which increases compatibility with a nucleoside. In order to raise compatibility, a means to enhance a canal interaction, an electrostatic interaction, a hydrogen bond, a pi-pi interaction (interaction of the magnetic fields which the ring current of an aromatic ring generates), and a CH/pi interaction (interaction of the magnetic field which the ring current of an aromatic ring and the electron of a methyl group generate) can be considered. Since it is predicted that Ser72 interacts with the base of a nucleoside most strongly, It seems that the substitution to Phe, Tyr, and Trp enhances a hydrophobic interaction and a pilpi interaction, the substitution to Va1, Ile, and Leu enhances a hydrophobic interaction and a CH/pi interaction, and the substitution to Glu and Asp reinforces an electrostatic interaction and a hydrogen bond. A hydrophobic interaction etc. may be enhanced by the substitution to other amino acid, especially the substitution to the amino acid which has a more nearly long-chain side chain. It is expected that a pilpi interaction will be formed between the aromatic ring of amino acid residue and the base of a nucleoside which were replaced also by replacing Leu16, Ser71, Ser73, and Glu104 by Phe, Tyr, and Trp. Formation of a hydrogen bond with the ribose of a nucleoside is expected by replacing Ile103 and Thr153 by more nearly long-chain hydrophilic residue. Since an opening will produce inside protein if it replaces by amino acid residue with small side chains [151 / which was located near the nucleoside binding site and has been buried in the inside of protein / Thr], such as Ser, Ala, and Gly, The pliability of a nucleoside binding site is expected whether to be able to take increase and the conformation which was suitable by combination with a nucleoside. Although Leu140 has separated not less than 10A from Ser72, it is located in the nearest to a phosphate bond part in the spacial configuration of a reaction intermediate analog. Therefore, when replacing this residue, the structure around a phosphate bond part in reaction intermediate changed, and it was thought by extension that influence also attained to the structure of a nucleoside binding site and fluctuation. If it replaces without Lys which has bulkier Phe and positive charge for this residue, and Glu which has a negative charge, it is hoped that compatibility with a nucleoside may change. The above-mentioned variation is introduced to G74 D/I153T variant EB-AP at the beginning. However, the enzyme variant made into the object of introduction is not restricted to G74 D/I153T variant EB-AP. For example, it is also possible to introduce variation into 10 residue substitution variant EB-AP. Variation is carried out to introducing to G74 D/I153T variant EB-AP. In this case, the enzyme variant produced turns

into 3 residue substitution enzyme variant. Details were shown in Example 9.

(6) Produce the plasmid which contains the gene which encodes variant EB-AP by the PCR method. A plasmid is introduced into Escherichia coli (Escherichia coli) JM109, and variant EB-AP is made to produce. Km value which becomes an index of compatibility to the inosine of variant EB-AP, and the phosphoryl-group-transfer activity which changes inosine into 5'-inosinic acid are measured, and the performance of variant EB-AP is evaluated. It is thought that it depends for the quantity of production of nucleoside 5'-phosphoric acid on Km value much. Pyrophoric acid reacts to EB-AP, phosphoric acid ion breaks away, and if a water molecule attacks this after the reaction intermediate of the form in which the phosphate group carried out the covalent bond to EB-AP is formed, a phosphate group will separate (phosphatase reaction). It means that pyrophoric acid was consumed vainly, without generating nucleoside 5'-phosphoric acid. On the other hand, if a nucleoside attacks reaction intermediate, a phosphate group will form a nucleoside and phospho monoester binding, and the generated nucleoside 5'-phosphoric acid will secede from EB-AP (phosphoryl-transfer reaction). It means that pyrophoric acid was utilized for nucleoside 5'-phosphoric acid generation. That is, if water and a nucleoside scramble for reaction intermediate, water wins and a nucleoside will win in phosphatase activity, phosphoryl-group-transfer activity will be demonstrated. If the compatibility over EB-AP of a nucleoside goes up (i.e., if Km value falls), a possibility that a phosphoryl-transfer reaction will be performed will become high. If the hydrophobicity near a phosphate bond part is improved and water becomes difficult to approach, phosphatase activity will become weaker and phosphoryl-group-transfer activity will become strong relatively. Details were shown in Example 10. (7) Conduct the experiment which produces 5'-inosinic acid from inosine using Escherichia coli JM109 which introduced the plasmid containing the variant EB-AP gene in which Km value fell to and phosphoryl-group-transfer activity went up. A reaction is performed at 30 ** for 45 hours, and aging of 5'-inosinic acid quantity of production is monitored. Details were shown in Example 12. (8) If the variation part in which Km is reduced is found out, by combining two or more residue, the compatibility over a nucleoside can improve further and the enzyme variant which became high [productivity] can be produced. Two or more mutation sites can be introduced by repeating sitespecific mutation cumulatively. If the primer from which the portion of the base which encodes the amino acid residue which introduces variation in the case of introduction of site-specific mutation became a mix base is used, the library of a variant gene where specific amino acid residue was replaced by all the amino acid can be created. If the primer of a mix base is used for two or more parts and variation is introduced, the library of the variant gene which encodes the enzyme variant of various sorts dramatically can be created. Thus, the method of selecting the variant which introduced the library of the built gene into Escherichia coli and with which the amino acid substitution of high activity was combined from the library made to reveal is also effective. [0012]If it is an enzyme with the space which the same active site and nucleoside as EB-AP can combine also except EB-AP, there is potential which can be used for production of nucleoside 5'phosphoric acid. An active site has indispensable amino acid residue with activity, and must be arranged by the space position relation with suitable them. In EB-AP, Lys115, Arg122, His150, Arg183, and His189 are indispensable with activity, and it is possible to specify space position relations with the distance between Calpha of these 5 residue. In this invention, since three EB-AP crystal structures, a wild type, G74 D/I153T variant, and a reaction intermediate analog, were determined, Calpha interatomic distance of the activity residue in each structure was measured, and Table 1 was created. Since each distance distribution of Table 1 had the width which is about 1A, when beyond a distance (Table 1, a minimum) shorter 1A than the shortest distance was below a distance (Table 1, a maximum) longer 1A than longest distance, I thought that the active site called for could be formed. The physical relationship of five residue was shown in drawing 1 with the distance between Calpha of the maximum and the minimum. In MM-AP which is a relative enzyme of EB-AP, it was shown in Example 15 that it was checked that all the interatomic distances between activity residue have fitted in the range specified from the spacial configuration of EB-AP. By this example, although the example of G72 D/I151T variant instead of a wild type was shown, it is thought that there is no big difference to the spacial configuration of an active site at the same wild type and variant of an enzyme. This guess is supported by that the structure of the active site of the wild type of EB-AP and G74 D/I153T variant is fundamentally the same (refer to Table 1). In order to

phosphorylate a nucleoside and to change into nucleoside 5'-phosphoric acid, just the active site that comprises the 5 above-mentioned residue is insufficient, and a nucleoside must be able to combine with a suitable position in it. In EB-AP, a grooved space suitable for a nucleoside joining near the binding site of a phosphate group together exists in the molecule surface. [(drawing 3): It can display on computer graphics (CG) using drawing 10 in which the atomic coordinates of which attachment was done are shown - drawing 45.]This slot is specified as a space surrounded by 4 residue of Leu16, Ser72, Glu104, and His189. Even if it has an active site, the enzyme without the suitable space which a nucleoside combines is unsuitable as a nucleoside 5'-phosphoric acid production enzyme. [0013]

[Table 1]

		野生型	G74D/I153T 変異型	反応中間体 アナログ	下限	上限
Lys115	Argl 22	11.6Å	11.6A	11.4Å	10.4Å	12.6A
	Hisi50	12.4Å	12.3Å	12. 8A	11.3A	13.8A
	Arg183	15. 4Å	16.3Å	15.5A	14.5A	17.4A
	His189	12. 6Å	12.1Å	11.7Å	10.7A	13. 6A
Arg1 22	His150	13. 2Å	13.6A	14. 2 Å	12. 2Å	15. 2.A
	Argl83	10.4Å	10.5A	10.8A	9.4A	11.8Å
	His189	5. 6.A	5.5A	5. 7 Å	4.5Å	6.7A
His150	Argi 83	8.4A	8.8Å	7.7Å	6.7A	9. 8.A
	H15189	9. 8Å	10.0Å	10.0Å	8. 8 A	11.0A
Arg183	Nis189	5. 5.A	5.8A	5.7A	4.5A	6.8Å

[0014] This invention provides preferably the substitution to other amino acid of Ser72, and Phe, Tyr, Trp, Val, Leu, Glu, Asp, Gln, Met, Thr, Arg and variant EB-AP that performed substitution to any one amino acid of Lys. The residue (residue number: 16, 70–71,73–76 and 100, 102–104, 106–108, 115, 148–154, 183) in Ser72 to 10A has a dramatically high possibility of interacting with a nucleoside, Variant EB-AP which performed substitution to other amino acid of these amino acid residue is provided. It includes here not only when amino acid being artificially replaced by substitution, but selecting other acid phosphatase belonging to the same enzymatic family as EB-AP which substitution produced in the nature. However, variant EB-AP containing mutational sites other than the above-mentioned amino acid residue can also provide this invention.

[0015]Also in other acid phosphatase belonging to the same enzymatic family as EB-AP, if amino acid variation of homologous is given, it is possible to use for manufacture of nucleoside 5'-phosphoric acid. However, the amino acid residue of EB-AP is not necessarily equivalent to the amino acid residue of the same number in other acid phosphatase. For example, Ser72 of EB-AP corresponds to Ala70 in MM-AP. Matching of the amino acid residue of two different protein, If the homology of both amino acid sequence is not less than about 20% and it is alignment (Sequence Alignment) of amino acid sequences, and about 20% or less, it will become clear by alignment (Threading) of the threedimensional structure and an amino acid sequence. The former can perform the latters, such as BLAST, by programs, such as INSIGHT II. Amino acid sequence alignment of EB-AP and Enterobacter aerogenes (Enterobacter aerogenes) origin acidity HOFATAZE (EA-AP) using BLAST was shown in Example 14. BLAST should just obtain the file in accordance with the computer used among the files which exist in /blast/executable from ncbi.nlm.nih.gov using FTP. Details are described by http://genome.nhgri.nih.gov/blastall/blast#install about operation information. [0016]Although attained by the improvement in compatibility with a nucleoside in many cases, it is attained by the shift of optimal pH, thermal stability improvement, etc. besides it, and deals in the improvement in nucleoside 5'-phosphoric acid productivity. The shift of optimal pH can be attained by changing pK of activity residue. [Protein engineering (Protein Engng.), the 1st volume, the 383-388th page (1998)] .Substitution to the glycine residue of the residue in which thermal stability improvement takes introduction of proline residue, and a counterclockwise twining helical structure [Protein

engineering, the 6th volume, the 85–91st page (1993)] The opening inside protein is filled. [Biochemistry (Biochemistry), the 32nd volume, the 6171–6178th page (1993)] ******** achievement is possible. It is effective in order to produce the variant whose nucleoside 5'-phosphoric acid productivity the compatibility of this spacial configuration of the nucleoside improved and improved, as explained to details above, but. This spacial configuration is effective not only in the compatibility over the nucleoside of an enzyme but changing compatibility with a phosphate donor. This enzyme can use various phosphoric ester compounds, such as polyphosphoric acid (salt), phenylphosphoric acid (salt), acetyl phosphate (salt), and carbamyl phosphate (salt), as a phosphate donor as indicated to JP,9–37785,A, but. It is possible to extend the substrate specificity of a phosphate donor or to raise the capacity factor of phosphoric acid by designing the variation which increases compatibility with a phosphoric ester compound in a way similar with having designed the variation which increases compatibility with a nucleoside.

[0017]

[Example]Hereafter, although an example explains this invention still more concretely, this invention is not limited to these examples.

[0018] Example 1 It crystallized using steamy diffusion with the crystallization hanging drop method of wild type EB-AP. 20mM buffer solution (pH 8.0) of sodium phosphate containing wild type EB-AP (concentration of 10mg/ml), the 100mM buffer solution of the trischloride containing the polyethylene glycol 400 of 45 (w/v %) -- an equivalent amount -- every (each 7-10microl). the well which filled 100mM buffer solution 500mul of the trischloride which carries out dropping mixing on the cover glass [SHIRIKONAIZEISHON / cover glass], and contains the polyethylene glycol 400 of 45 (w/v %) -- it covered so that a mixed liquor drop might fish and fall on (well), and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, two weeks afterward, it grew up to be a crystal of the shape of a hexagonal prism of a measurable size (about 0.3x0.3x1.2 mm) from one week. On the occasion of Xray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). This crystal needed to care about the following point on handling. 1) Since a crystal collapsed very easily by contacting a container and tools from a drop (droplet) when taking out a crystal, the crystallization gestalt of the sitting dropping method could not be used (a crystal grows), but used the hanging drop method described here. 2) At ordinary temperature measurement, since a crystal deteriorated during measurement and resolution fell gradually, it needed to be measured under the low temperature service. Time until it mounts a crystal on a stage was shortened as much as possible, and it devised so that it might not expose to air. Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group was set to P6322 and the grating constant became a=b=124.4A and c= 97.7 A. If it assumes that one subunit of the molecular weight 25000 is included in an unsymmetrical unit, the moisture content of a crystal will be 72%. [0019]It crystallized using steamy diffusion with the crystallization hanging drop method of example 2 G74 D/I153T variant EB-AP. 20mM buffer solution (pH 8.0) of the trischloride containing G74 D/I153T enzyme variant (concentration of 20mg/ml), 20mM buffer solution of the trischloride containing the polyethylene glycol 400 of 38 (w/v %) -- an equivalent amount -- every (each 5microl). Dropping mixing was carried out on the cover glass [SHIRIKONAIZEISHON / cover glass], it covered so that a mixed liquor drop might fish and fall on the well which filled 20mM buffer solution 500mul of the trischloride containing the polyethylene glycol 400 of 38 (w/v %), and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, one week afterward, it grew up to be a plate crystal of a measurable size (about 0.7x0.4x0.2 mm). On the occasion of X-ray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group became P2 12 12 1, and the grating constant became a= 138.0 A, b= 168.3 A, and c= 58.2 A. If it assumes that one hexamer molecule of the molecular weight 150000 is included in an unsymmetrical unit, the moisture content of a crystal will be 64%.

[0020]Example 3 It crystallized using the cocrystal-ized method for having used steamy diffusion by the crystallization sitting dropping method of the complex (reaction intermediate analog) of wild type

EB-AP and molybdic acid. 20mM buffer solution (pH 8.0) of sodium phosphate containing wild type EB-AP (concentration of 10mg/ml), the 100mM buffer solution of the trischloride containing the polyethylene glycol 400 of 40 (w/v %), and sodium molybdate of 1mM — an equivalent amount — every (each 15microl). Dropping mixing was carried out and it settled on the hollow of the bridge installed in the well which filled 500micro of 100mM buffer solution (pH 8.0) I of the trischloride containing the polyethylene glycol 400 of 40 (w/v %) at 20 **. The crystal deposited two to three days afterward, and, two weeks afterward, it grew up to be a crystal of the shape of a diamond—shaped rice cake of a measurable size (about 0.3x0.3x0.3 mm) from one week. On the occasion of X-ray-data measurement, the crystal was moved to the 100mM buffer solution (pH 8.0) of the trischloride containing the polyethylene glycol 400 of 50 (w/v %). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group was set to P3₁21 and the grating constant became a=b=86.6A and c= 205.3 A. If it assumes that three subunits of the molecular weight 25000 are included in an unsymmetrical unit, the moisture content of a crystal will be 58%.

[0021]Example 4 The X diffraction data to a maximum of 1.9A of crystal-structure-analysis resolution data of wild type EB-AP was measured. In ordinary temperature, since the damage by the exposure of X-rays was intense, the crystal measured by cooling quickly at -173 **. The Shigehara child derivative was screened by dipping a crystal into the solution of heavy metal salt. The diffraction data of the Shigehara child derivative crystal was obtained using Rigaku R-AXIS IIc. K2PtCl4 found out giving the good Shigehara child same type crystal from the difference Fourier figure with native data. By using the program RSPS, the coordinates of the only platina binding site of K₂PtCl₄ were searched for. It asked for the phase which carries out elaboration of these coordinates by the program MLPHARE, and is calculated. It asked for five mercury binding sites of the 2nd Shigehara child derivative KHgI₄-KI using this phase. After carrying out elaboration of the Shigehara child parameter of both $\rm K_2PtCl_4$ and $\rm KHgI_4$ -KI simultaneously using MLPHARE, using program DM, solvent smoothing and histogram matching were performed and the phase was improved. Incidentally, anomalous dispersion data was also used about K2PtCl4. The electron density map calculated using this good phase was dramatically clear, and was able to fit almost all amino acid residue finely. The first model was built using the program QUANTA on the electron density map created with 2.8A resolution, and performed structure refinement using program X-PLOR. Electron density was not observed and 6 residue in the end of N, the 135 to 136th residue, and 1 residue in the end of C were not able to determine structure uniquely. The final model (drawing d - drawing 6) by which elaboration was carried out with 1.9A resolution contains all the 222 in 231 residue residue, 236 water molecules, and one molecule of sulfate ion. Sulfate ion originates in the ammonium sulfate used in refining processes. It is considered a match by the phosphate bond part of an active center.

The crystallographic reliability factor (R factor) using reflection of 8-1.9A resolution became 21.5%. The average temperature factor became 26A² about the protein atom, and became 45A² about the water molecule. When Ramachandran plot was created using the program PROCHECK, it was shown in the field where 93% of residue other than a glycine is the most preferred that 7% is located in a field desirable next. One subunit is contained in an unsymmetrical unit and a hexamer is formed of crystallographic symmetry. Atomic coordinates were shown in drawing 10 - drawing 45. [0022]Drawing 4 is a CG photograph which shows the crystal structure of the hexamer molecule of EB-AP. The flow of alpha carbon atom was displayed with the ribbon model. The sulfate ion which marks an active center was displayed with the ball model. Drawing 5 is a CG photograph which shows the crystal structure of the subunit of EB-AP. The flow of alpha carbon atom was displayed with the ribbon model. The sulfate ion which marks an active center was displayed with the ball model. Drawing 6 is a figure showing the active site architecture of EB-AP. Sulfate ion was shown in the center. The dotted line showed the hydrogen bond. Drawing 10 is a figure showing the crystallography data (1) of the structure of EB-AP. Drawing 11 is a figure showing the crystallography data (2) of the structure of EB-AP. Drawing 12 is a figure showing the crystallography data (3) of the structure of EB-AP. Drawing 13 is a figure showing the crystallography data (4) of the structure of EB-AP. Drawing 14 is a figure showing the crystallography data (5) of the structure of EB-AP. Drawing 15 is a figure showing the crystallography data (6) of the structure of EB-AP. Drawing 16 is a figure showing the crystallography data (7) of the structure of EB-AP. Drawing 17 is a figure showing the crystallography data (8) of the structure of EB-AP. Drawing 18 is a figure showing the crystallography data (9) of the structure of EB-AP. Drawing 19 is a figure showing the crystallography data (10) of the structure of EB-AP. Drawing 20 is a figure showing the crystallography data (11) of the structure of EB-AP. Drawing 21 is a figure showing the crystallography data (12) of the structure of EB-AP. Drawing 22 is a figure showing the crystallography data (13) of the structure of EB-AP. Drawing 23 is a figure showing the crystallography data (14) of the structure of EB-AP. Drawing 24 is a figure showing the crystallography data (15) of the structure of EB-AP. Drawing 25 is a figure showing the crystallography data (16) of the structure of EB-AP. Drawing 26 is a figure showing the crystallography data (17) of the structure of EB-AP. Drawing 27 is a figure showing the crystallography data (18) of the structure of EB-AP. Drawing 28 is a figure showing the crystallography data (19) of the structure of EB-AP. Drawing 29 is a figure showing the crystallography data (20) of the structure of EB-AP. Drawing 30 is a figure showing the crystallography data (21) of the structure of EB-AP. Drawing 31 is a figure showing the crystallography data (22) of the structure of EB-AP. Drawing 32 is a figure showing the crystallography data (23) of the structure of EB-AP. Drawing 33 is a figure showing the crystallography data (24) of the structure of EB-AP. Drawing 34 is a figure showing the crystallography data (25) of the structure of EB-AP. Drawing 35 is a figure showing the crystallography data (26) of the structure of EB-AP. Orawing 36 is a figure showing the crystallography data (27) of the structure of EB-AP. Drawing 37 is a figure showing the crystallography data (28) of the structure of EB-AP. Drawing 38 is a figure showing the crystallography data (29) of the structure of EB-AP. Drawing 39 is a figure showing the crystallography data (30) of the structure of EB-AP. Orawing 40 is a figure showing the crystallography data (31) of the structure of EB-AP. Drawing 41 is a figure showing the crystallography data (32) of the structure of EB-AP. Or awing 42 is a figure showing the crystallography data (33) of the structure of EB-AP. Drawing 43 is a figure showing the crystallography data (34) of the structure of EB-AP. Drawing 40 is a figure showing the crystallography data (35) of the structure of EB-AP. Drawing 45 is a figure showing the crystallography data (36) of the structure of EB-AP.

[0023]Example 5 Since it exceeds 100mM, Km value of compatibility to wild type EB-AP and EB-AP of the guess inosine of the bond form model of 5'-inosinic acid is not so high as it can determine a bond form by X ray crystal structure analysis. After actually soaking the compound used as inhibitor of EB-AP, such as glucose 6-sulfate and adenosine thio monophosphate, into the crystal of wild type EB-AP, collected X diffraction data, and created the electron density map, but. The electron density corresponding to these compounds was not observed. Then, we decided to guess the bond form of 5'-inosinic acid and EB-AP (what is called a docking study) using computer graphics. The program used QUANTA. Among the crystal structure, since sulfate ion was found out in the center of an active site, the phosphate group of 5'-inosinic acid was piled up here. Since it was known that G74D and the variation of I153T will reduce Km value to EB-AP of 5'-inosinic acid, it judged that 5'-inosinic acid was combined with the place in which a long distance is not from G74 and I153, and the position of 5'-inosinic acid was decided. The atom which constitutes 5'-inosinic acid, and the atom which constitutes EB-AP were kept from colliding mutually in that case. In this way, in the built model, if I153 is set to T, the gamma acid matter atom of the side chain of threonine and 2' hydroxyl group of the ribose of inosine which were replaced will form a hydrogen bond. When an electrostatic potential indication of EB-AP is given using the program GRASP, the inosine base which is tinged with positive charge is interacting with the field which is tinged with the negative charge on the EB-AP molecule surface.

It was suggested that a model will seemingly be reasonable.

[0024] The ratio of phosphoryl-group-transfer activity [as opposed to phosphatase activity in crystal-structure-analysis G74 D/I153T variant EB-AP of example 6 G74 D/I153T variant EB-AP] is increasing.

In connection with it, the production capacity of nucleoside 5'--phosphoric acid is also improving. It is considered as a cause that it improved that this Km value with a nucleoside fell, i.e., compatibility with a nucleoside. When determining the crystal structure of this variant EB-AP and comparing with the crystal structure of wild type EB-AP, it was expected that the molecular mechanism of the improvement in compatibility with a nucleoside would be clarified. At ordinary temperature, the X diffraction data to a maximum of 2.4A resolution data was measured. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and one molecule of a hexamer is contained in an unsymmetrical unit. Then, it analyzed with the molecular replacement method using the program amore by making hexamer structure of wild type EB-AP into a search model. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translation search. The correct answer of both searches appeared as top peaks. When elaboration was performed by using a molecule as a rigid body, the R factor fell to 37.3%. Then, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of 19.9% of the R factor was obtained in 10-2.4A resolution. When the coupled models of 5'-inosinic acid and G74 D/I153T variant EB-AP were created by the same method as Example 5, it was expected that the gamma acid matter atom of the side chain of replaced Thr153 forms the hydroxyl group and hydrogen bond of a ribose of inosine. By comparing a temperature factor showed that the direction of G74 D/I153T variant EB-AP was large compared with a wild type in fluctuation of the loop containing Asp74 to which one more substitution was performed. Although it is expected that this loop interacts with the base of inosine, when fluctuation became large, a possibility of becoming easy to carry out combination with a base is suggested.

[0025]Example 7 In the enzyme reaction of crystal-structure-analysis EB-AP of the complex (reaction intermediate analog) of wild type EB-AP and molybdic acid, first, monoester phosphate combination is cut and a phosphate group forms His189 of activity residue, and a covalent bond. The enzyme molecule of this state is called reaction intermediate. Promptly, reaction intermediate receives the attack by water or alcohol, and, as a result, phosphoric acid ion secedes from it. If water attacks, phosphatase activity will be demonstrated, and if alcohol attacks, phosphoryl-group-transfer activity will be demonstrated. Anyway, reaction intermediate is unstable and it is impossible to determine the structure by X ray crystal structure analysis. However, since that (reaction intermediate analog), as for, molybdic acid carried out the covalent bond to His189 instead of phosphoric acid does not receive the attack by water, it exists in stability. In a phosphoryl-transfer reaction, a phosphate acceptor combines with reaction intermediate and monoester phosphate combination is formed. Therefore, it is more suitable to use reaction intermediate structure rather than isolation mold structure in the purpose of presuming a bond form with a nucleoside. Crystal structure analysis of the reaction intermediate analog was conducted in order to perform a docking study with reaction intermediate and a nucleoside. At ordinary temperature, the X diffraction data to a maximum of 2.4A resolution data was measured. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and three half of a hexamer, i.e., subunits, is included in an unsymmetrical unit. Then, with the threefold rotation axis, the trimer structure where each other was connected was created and it was considered as the search model of the molecular replacement method. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translationsearch. The correct answer of both searches appeared as top peaks. When elaboration was performed by using a molecule as a rigid body, the R factor fell to 42.4%. Then, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of 22.3% of the R factor was obtained in 8-2.4A resolution. Three molecule half of a hexamer, i.e., subunits, is included in an unsymmetrical unit.

[0026] The bond form model was built using QUANTA on the guess computer graphics of the bond form model of example 8 EB-AP reaction intermediate and inosine (drawing 3). Molybdic acid was transposed to phosphoric acid as it was. Inosine was placed near the nucleoside portion of 5'-inosinic acid in the bond form model of wild type EB-AP and 5'-inosinic acid. However, although it is natural, since inosine does not have monoester phosphate combination, flexibility is higher [inosine] than making 5'-inosinic acid dock. Therefore, inosine tuned the position of inosine finely and considered it

as the bond form model so that it might combine with the molecule surface of EB-AP in the more desirable state. We decided to use this model for the design of subsequent enzyme variants. [0027]Example 9 According to the model built in design example 8 of variant EB-AP which aimed at the improvement in compatibility with a nucleoside, a possibility that the side chain of Ser72 would interact with the base of inosine was suggested. When this residue was replaced by aromatic amino acid, such as phenylalanine, tyrosine, and tryptophan, the pi-pi interaction arose between the aromatic ring and the nucleoside base, and it was predicted that the compatibility over EB-AP of a nucleoside improves. Similarly, if it replaces by branched chain hydrophobic amino acid, such as valine, leucine, and isoleucine, When replaced by the amino acid which the CH/pi interaction arose between the branched chain hydrophobic group and the nucleoside base, and is tinged with negative charges, such as glutamic acid and aspartic acid, it paid well to the positive charge of a nucleoside base, and electrostatic, and improvement in compatibility was expected. Then, in order that phosphoryl-group-transfer activity may improve further the phosphoryl-group-transfer activity of relatively increasing G74 D/I153T variant EB-AP rather than phosphatase activity, We decided to produce S72F of this variant EB-AP, S72Y, S72W, S72V, S72E, and an S72D variant. We decided to also produce the variant which replaced S72 by other amino acid. Incidentally, these variants serve as 3 residue substitution variant EB-AP.

[0028] In order to build nine kinds of variant EB-AP for revealing Example 10Ser72 by production Escherichia coli JM109 of 3 residue substitution variant EB-AP replaced to other amino acid, Plasmid pEPI340 containing a G74 D/I153T variant EB-AP gene was used as a mold of the site-directedmutagenesis method using PCR. pEPI340 is the plasmid which added the variation of G74 D/I 153T to plasmid pEPI305. A deer is carried out and the base sequence of these plasmids pEPI305 and pEPI340 is specified in Table 12 of the paragraph number (0143) of JP,10-201481,A. It is named AJ13144 and the international deposit of the stock which made plasmid pEPI305 hold to Escherichia coli JM109 is carried out to National Institute of Bioscience and Human-Technology, the Ministry of International Trade and Industry, as FERM BP-5423. [Refer to the paragraph number (0105) of the above-mentioned publication before examination - (0110) a statement.] .Variation uses "a quick change site-directed-mutagenesis kit (Quickchange Site-Directed Mutagenesis Kit)" of Stratagene (Stratagene) (United States), and according to the protocol of the manufacturer, It introduced using the primer (drawing 7, array numbers 11-61 of an array table) corresponding to various enzyme variants. Escherichia coli XL-1 was transformed using the output of an PCR reaction. The transformed cell was smeared on L agar-medium plate containing 100 microl./ml ampicillin, and it incubated at 37 ** for 16 hours. The generated colony was extracted, and it cultivated, shaking overnight by L culture medium containing 100 microl./ml ampicillin. FlexiPrep Kit of Pharmacia Corp. (Sweden) was used after collecting biomasses by centrifugal separation from culture medium, and the plasmid was extracted according to the protocol of the manufacturer. The base sequence which encodes 3 residue substitution variant EB-AP in some numbers was checked by DNA sequence analysis. Composition of the primer set shown in drawing 7 was entrusted to Japanese Bio-Service. [0029]Example 11 Escherichia coli JM109 which introduced the plasmid containing the phosphorylgroup-transfer activity of variant EB-AP and the measurement various 3 residue substitution variant EB-AP genes of a velocity constant was inoculated into 50 ml of L culture media containing 100 microl./ml ampicillin, and it cultivated at 37 ** for 16 hours. Biomasses were collected by centrifugal separation from culture medium, and it was suspended to 3 ml of 25mM phosphoric acid buffers (pH 7.0), and crushed by performing ultrasonication for 20 minutes at 4 **. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. It checked by SDS-PAGE that each EB-AP3 residue substitution enzyme variant was revealed. The expression amount was about 20% of quality of total protein. The phosphoryl-group-transfer activity of the cellfree extract was measured on condition of the following. 2mM inosine, 100mM sodium pyrophosphate. a 100mM acetic acid buffer (pH 4.0), and the reaction mixture (1 ml) containing the cell-free extract of 100microl were incubated for 10 minutes at pH 4 and 30 **. After adding 1N chloride 200mul and stopping a reaction, under [a fixed quantity / inosinic acid / which was generated except for precipitate by centrifugal separation / 5'-]. The relative activity set to 1 showed 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP which set phosphoryl-group-transfer activity of 3 residue substitution variant EB-AP as the object which introduces variation in some numbers was

used. Then, Km value to the inosine in the phosphoryl-transfer reaction of 3 residue substitution variant EB-AP was measured on condition of the following in some numbers. The reaction mixture (1 ml) containing 100mM sodium pyrophosphate, a 100mM acetic acid buffer (pH 4.0), 10-100mM inosine, and the cell-free extract of 100microl was incubated for 10 minutes at pH 4 and 30 **. After adding 1N chloride 200mul and stopping a reaction, under [a fixed quantity / inosinic acid / which was generated except for precipitate by centrifugal separation / 5'-]. Km value was computed by Hanes-Woolf plot. A result is shown in Table 2. [0030]

[Table 2]

	Km値	リン酸転移活性
S72F/G74D/I153T	20mM	2.80
S72Y/G74D/1153T	25mM	2,04
S72W/G74D/1153T	30mW	1.71
S72D/G74D/1153T	33mM	1.59
S72V/G74D/I153T	40mM	2.46
S72E/G74D/I153T	40mM	3.19
S72N/G74D/I153T	46mM	1.94
S72T/G74D/I153T	50mM	1.91
S72L/G74D/I153T	57mM	2, 24
S72R/G74D/I153T	59mM	1.99
S72Q/G74D/I153T	77mM	2.42
S72K/G74D/I153T	78mX	1.53
S72P/G74D/1153T	109mW	1.34
S72A/G74D/I153T	115mM	0.78
S72N/G74D/1153T	124mM	0.43
S72G/G74D/I153T	137mM	0.43
S72H/G74D/I153T	n.d.	n. d.
G74D/1153T	100mM	1.00
10残基置換変異型	40mM	1.44

[0031]Km value to the inosine of all the variants (S72F, S72Y, S72W, S72V, S72L, S72E, S72D) predicted that compatibility with inosine will improve by the pi-pi interaction, the CH/pi interaction. and an electrostatic interaction in Example 9, It fell compared with the thing of G74 D/I153T variant EB-AP which does not carry out variation introduction, and the compatibility over inosine improved. Improvement was found also about phosphoryl-group-transfer activity. The variant of the improvement which introduced S72F especially was remarkable in Km value, phosphoryl-grouptransfer activity, and both sides. The aromatic ring and inosine base of phenylalanine carry out pi-pi interaction by suitable physical relationship, and it is surmised that improvement in compatibility was achieved. Km value of S72M, S72T,R [S72], S72Q, and S72K variant also fell. It is thought that some desirable interactions, such as a hydrophobic interaction and a hydrogen bond, arose between these amino acid residue and a nucleoside base. Incidentally, a gene was not able to be produced about S72I. Since there was a danger of making the mistaken SS linkage forming, S72C was not produced. High performance chromatography (HPLC) analyzed 5'-inosinic acid on condition of the following. column: -- Cosmosil 5C18-AR (4.6x150 mm) Nacalai Tesque, Inc. product mobile phase: -- 5mM potassium phosphate buffer (pH 2.8) / methanol =95/5 rate-of-flow: -- 1.0 ml/min temperature: -room temperature detection: -- UV245nm [0032]Production G74 D/I153T variant of 5'-inosinic acid using Escherichia coli JM109 which introduced the example 12 S72 F/G74 D/I153T variant EB-AP gene, Escherichia coli JM109 which introduced the plasmid containing 10 residue substitution variant and an S72 F/G74 D/I153T variant EB-AP gene was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG 1mM, and it cultivated at 37 ** for 16 hours, 12 g/dl of pyrophoric acid and 6 g/dl of inosine are dissolved in an acetic acid buffer (pH 4.0), The reaction was performed at 30 ** for 24 hours, maintaining [added the biomass of Escherichia coli JM109 which

introduced each of above-mentioned variant EB-AP genes into this so that it might become 100 mg/dl by dry cell weight, and] pH to 4.0. The result of having measured the quantity of generated 5'-inosinic acid was shown in Table 3. As for the generated inosinic acid, the byproduction of 2'-inosinic acid and 3'-inosinic acid was not accepted at all only from 5'-inosinic acid. Although 7.5 g/dl of 5'-inosinic acid carried out generation accumulation at the reaction using Escherichia coli JM109 which introduced the plasmid containing a G74 D/I153T variant EB-AP gene, even if it developed reaction time, accumulation did not increase more than it. At the reaction using Escherichia coli JM109 which introduced the plasmid containing a 10 residue substitution variant EB-AP gene, accumulation improved and 5'-inosinic acid which is 12.1 g/dl carried out generation accumulation. It designed based on the spacial configuration, and at the reaction using Escherichia coli JM109 which introduced the plasmid containing the built S72 F/G74 D/I153T variant EB-AP gene, productivity improved further and 5'-inosinic acid which is 13.2 g/dl carried out generation accumulation. [0033]

[Table 3]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
G74D/I153T	7. 5
10残基置換変異型	12. 1
S72F/G74D/I153T	13. 2

[0034] Measurement S72F variation of the phosphoryl-group-transfer activity of 3 residue substitution variant EB-AP and a velocity constant which introduced example 13 L16W, S71W, S73W, E104F, and E104W variation, Since it was thought that compatibility with inosine was raised by the pipi interaction, it searched for other amino acid residue which can plan a pi-pi interaction with an inosine base by substitution to aromatic ring amino acid on computer graphics. As a result, a possibility that the aromatic ring replaced by L16W, S71W, S73W, E104F, and E104W variation would interact with an inosine base was suggested. Then, a method which described these five sorts of 3 (G74 D/I153T variant EB-AP was used as base) residue substitution variant EB-AP in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 8.) It produced with the array numbers 62-76 of the array table, and phosphoryl-group-transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 4. Although phosphoryl-group-transfer activity fell also in which enzyme variant, Km value fell with all the enzyme variants, and it was suggested that the compatibility with inosine improved. Although Leu16 had separated 10A from Ser72 an interaction with inosine is predicted to be (in distance between Calpha), even if separated to this extent, it was shown that an interaction with inosine is possible. Composition of the primer set shown in drawing 8 was entrusted to Japanese Bio-Service. [0035]

[Table 4]

	Km位	リン酸基転移活性
L16W/G74D/I153T	33mM	0.21
S71W/G74D/I153T	75mM	0.26
S73W/G74D/1153T	29mM	0.77
E104F/G74D/1153T	61mM	0.65
E104W/G74D/1153T	67mM	0. 26
G74D/1153T	100mM	1.00
10残基置換変異型酵素	40mM	1.44

[0036]Example 14. In production EB-AP of 5'-inosinic acid using Escherichia coli JM109 which introduced production and this gene of the variant enzyme gene of Enterobacter aerogenes

(Enterobacter aerogenes) origin acid phosphatase (EA-AP). It was presupposed that a variation [homologous / variation / three / of S72 F/G74 D/I 153T which raised the phosphoryl-group-transfer activity of inosine] is introduced into EA-AP. The result of having aligned the amino acid sequence of EB-AP and EA-AP using the program BLAST is shown in drawing 9. It was shown that Ser72/Gly 74-/Ile153 of EB-AP corresponds to Ala70/Gly 72-/Ile151 in EA-AP. Then, A70 F/G72 D/I151T variant EA-AP was produced by the method described in the Example 10. It carried out using Escherichia coli JM109 which introduced the plasmid containing a variant enzyme gene by the method which described production of 5'-inosinic acid in the Example 12 from inosine. A result is shown in Table 5. A70 F/G72 D/I151T variant EA-AP showed 5'-inosinic acid productivity equivalent to S72 F/G74 D/I153T variant EB-AP.

[Table 5]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
EA-AP A72F/G74D/1153T	13. 1
BB-AP S72F/G74D/I153T	13. 2

[0038] Drawing 9 is a figure showing the result of having performed amino acid sequence alignment of EB-AP and Enterobacter aerogenes origin acid phosphatase (EA-AP) by the program BLAST. The upper row is EB-AP and the lower berth is EA-AP. If it is similar amino acid residue even if the residue name is not the same, if both amino acid residue is the same, + will be displayed on the middle. The position of the 72nd residue (Ser72) of EB-AP was marked by [72]. Residue corresponding by EA-AP is Ala70.

[0039]Example 15 G72 D/I151T double mutant of MM-AP was crystallized using crystallization of Morganella MORUGANI origin acid phosphatase (MM-AP) origin G72 D/I151T enzyme variant, and steamy diffusion with a crystal-structure-analysis hanging drop method. The protein solution (concentration of 40mg/ml) concerned, and the polyethylene glycol 1000 of 25 (w/v %), 25mM ammonium sulfate, 125mM citrate buffer solution containing 25mM DTT (pH 4.8) an equivalent amount --- every (each 5microl). Carry out dropping mixing on the cover glass [SHIRIKONAIZEISHON / cover glass], and The polyethylene glycol 1000 of 25 (w/v %), 25mM ammonium sulfate, 125mM citrate buffer solution containing 25mM DTT (pH 4.8) It covered so that a mixed liquor drop might fish and fall on the well which filled 500microl, and it settled at 20 **. The crystal deposited 2 or 3 days afterward, and, one week afterward, it grew up to be a measurable size (about 0.4x0.4x0.3 mm). Using X-ray diffractometer R-AXIS IIc of Rigaku, X diffraction data was collected and the crystallographic parameter was determined. The space group became P2 12121 and the grating constant became a= 90.64 A, b= 119.74 A, and c= 136.14 A. The diffraction data to 2.6A resolution data was measured by 100K on Tsukuba and high energy research institute synchrotron radiation institution BL-6B. It was expected that estimate from the molecular weight of the volume of a unit cell, a space group, and an enzyme, and one molecule of a hexamer is contained in an unsymmetrical unit. Then, it analyzed with the molecular replacement method using the program amore by making hexamer structure of wild type EB-AP into a search model. In rotation search, the data of 10-4A resolution was used for the data of 10-3A resolution in translation search. The correct answer of both searches appeared as top peaks. After performing elaboration by using a molecule as a rigid body, it carried out by having repeated the structure correction on the graphics using QUANTA, and the structure refinement using X-PLOR, and the model of R factor 0.197 was obtained in 10-2.6A resolution. The distance between Calpha atoms of five activity residue (Lys113, Arg120, His148, Arg181, His187) shown in drawing 1 was shown in Table 6. In MM-AP which is a relative enzyme of EB-AP, it was checked that all the interatomic distances between activity residue have fitted in the range specified from the spacial configuration of EB-AP.

[0040]

[Table 6]

		G74D/1153T 交異型知-AP	下限	上限
Lys113	Arg120	11.3A	10.4Å	12.6A
	His148	12. 6Å	11.3Å	13.8A
	Arg181	16.3Å	14.5Å	17.4Å
	His187	12.5Å	10.7Å	13.6A
Arg120	His148	14. 0A	12. 2Å	15. 2Å
	Arg181	10. 9Å	9.4A	11.8A
	H1s187	6.1A	4.5Å	6.7Å
His148	Arg181	8.9A	6.7Å	9.8Å
	H1s187	10. 2Å	8.8A	11.0A
Arg181	His187	5.4A	4.5Å	6.8Å

[0041]In production of 10 residue substitution variant EB-AP which introduced example 16 A72F and A72E variation, and the measurement example 11 of phosphate transfer activity and a velocity constant, S72F to which Km value was reduced most, and the S72E variation which improved activity most were introduced into 10 residue substitution variant EB-AP. In 10 residue substitution variant EB-AP, since Ser72 is replaced by Ala, A72F and A72E variation will be introduced actually. If based on wild type EB-AP, 10 residue is replaced for both sides. A method which described these two sorts of variants in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 46.) It produced with the array numbers 77-82 of the array table. As a mold of the site-directed-mutagenesis method using PCR, plasmid pEMP370 (JP,9-37785,A, Example 19) containing a 10 residue substitution variant EB-AP gene was used. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 7. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity. Also in which enzyme variant, Km value fell notably. About phosphate transfer activity, it went up by A72E variation to having fallen by A72F variation.

[0042]

[Table 7]

	Km値	リン酸転移活性
A72F/10残基置换变吳型酵素	9mW	0.11
A72E/10残基置换变異型酵素	15mM	2.30
10残基置換変異型酵素	40mM	1.44

[0043]Production A72F / 10 residue substitution variant EB-AP of 5'-inosinic acid using Escherichia coli JM109 which introduced example 17 A72F / 10 residue substitution variant EB-AP, and A72E / 10 residue substitution variant gene. The production experiment of 5'-inosinic acid using Escherichia coli JM109 which introduced the included plasmid was conducted by the method described in the Example 12. A result is shown in Table 8. The accumulated dose of 5'-inosinic acid increased both variants.
[0044]

[Table 8]

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
A72F/10残基置換変異型	13.9
A72E/10残基置换变異型	13.9
10稅基份檢密異型	12.1

[0045]By measurement I103D variation of production of variant EB-AP which introduced example 18 I103D and T153N variation, phosphate transfer activity, and a velocity constant. It was suggested with the model of drawing 3 that replaced Asn forms the hydroxyl group and hydrogen bond of a ribose by that replaced Asp carries out an inosine base and an electrostatic interaction and T153N variation. Then, a method which introduced such residue into G74 D/I153T variant EB-AP, and described I103 D/G74 D/I153T variant EB-AP and G74 D/I153N variant EB-AP in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 47.) We decided to produce

with the array numbers 83–88 of an array table. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 9. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity. It was suggested that Km value fell and the compatibility of both variants with inosine improved although phosphoryl-group-transfer activity fell. [0046]

[Table 9]

The state of the s	Km値	リン酸転移活性
I103D/G74D/I153T	51 mM	0.09
G74D/1153N	38mM	0.18
G74D/I153T	100mM	1.00

[0047] Although production of variant EB-AP which replaced Example 19Leu140 by Phe, Glu, and Lys, and measurement Leu140 of phosphate transfer activity and a velocity constant have separated not less than 10A from Ser72, they are located in the nearest to a phosphate bond part in the spacial configuration of a reaction intermediate analog. Therefore, when replacing this residue, the structure around a phosphate bond part in reaction intermediate changed, and it was thought by extension that influence also attained to the structure of a nucleoside binding site and fluctuation. If it replaces without Lys which has bulkier Phe and positive charge for this residue, and Glu which has a negative charge, it is hoped that compatibility with a nucleoside may change. Variation decided that phosphate transfer activity introduces into high A72E / 10 residue substitution product in Example 16. A method which described these three sorts of variants in the Example 10 (the primer corresponding to each enzyme variant was shown in drawing 48.) It produced with the array numbers 89-97 of the array table. As a mold of the site-directed-mutagenesis method using PCR, the plasmid containing A72E / 10 residue substitution variant EB-AP gene was used. Phosphate transfer activity and a velocity constant were measured by the method described in the Example 11. A result is shown in Table 10. The relative activity which set 5'-inosinic acid generated amount when G74 D/I153T variant EB-AP was used to 1 showed phosphate transfer activity. [0048]

[Table 10]

	Km値	リン酸転移活性
A72E/L140F/10残基價換変異型酵素	9mM	1.66
A72E/L140K/10残基置換変異型酵素	78mM	0.07
A72E/L140E/10残基置換変異型酵素	322mM	0.16
A72E/10残基置換変異型酵素	15mM	2. 30

[0049]Km value fell [the variant which introduced L140F]. On the contrary, L140K and L140E variation raised Km substantially.

[0050]example 20 Enterobacter aerogenes . IFO. 12010 . Acid phosphatase of the Enterobacter aerogenes IFO12010 origin from the culture object of refining of origin wild type acid phosphatase and Escherichia coli JM109/pENP110 of example 24 statement of determination JP,10–201481,A of N-terminal-amino-acid arrangement. It refined, N-terminal-amino-acid arrangement was determined, and the amino acid sequence of maturation protein was determined. Escherichia coli JM109/pENP110 is the bacillus which introduced the acid phosphatase gene of the Enterobacter aerogenes IFO12010 origin into 109 shares of Escherichia coli JM, and produces this acid phosphatase. The amino acid sequence of the precursor protein expected from the base sequence of this acid phosphatase gene is arrangement shown in the array number 10 of an array table. 50 ml of nutrient media (pH 7.0) containing 1 g/dl of peptone, 0.5 g/dl of yeast extracts, and 1 g/dl of salt were put into a 500-ml Sakaguchi flask, and it heat-sterilized for 20 minutes at 120 **. One platinum loop of Escherichia coli JM109/pENP110 was inoculated into this, and shaking culture was carried out at 30 ** for 16 hours. The biomass which collected biomasses from culture medium by centrifugal separation was suspended to the 100mM potassium phosphate buffer (pH 7.0) of 100 ml, ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. The treating solution was

centrifuged and the cell-free extract was prepared except for the insoluble fraction. Ammonium sulfate was added so that it might become this cell-free extract with saturation 30%. After removing the precipitate generated by centrifugal separation, additional addition of the ammonium sulfate was carried out so that it might become digestive liquor with saturation 60%. Centrifugal separation recovered the generated precipitate and it dissolved in the 100mM potassium phosphate buffer. After dialyzing this crude enzyme liquid 3 times to 500 ml of 100mM potassium phosphate buffers (pH 7.0), It charged in DEAE-TOYOPARU 650M column (phi3.0x10.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0), and 20mM potassium phosphate buffer (pH 7.0) washed. Since phosphate transfer activity suited the bypassing fraction, the fractions concerned were collected. Ammonium sulfate was added so that it might become this activity fraction with saturation 35%, and this was made to stick to the butyl-Toyopearl column (phi3.0x7.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0) which contains saturated ammonium sulfate 35%. This was eluted by the linear concentration gradient of the saturated potassium phosphate buffer (pH 7.0) 20% from saturation 35%. CM-TOYOPARU equilibrated with 10mM potassium phosphate buffer (pH 6.0) after collecting activity fractions and dialyzing to 10mM potassium phosphate buffer (pH 6.0) 1L. It was made to stick to a column (phi3.0x7.0cm). This was eluted by the linear concentration gradient of the potassium phosphate buffer (pH 6.0) containing 300mM potassium chloride from 0mM. These activity fractions were collected. The above operation refined the enzyme in which phosphate transfer activity is shown about 5 times with the recovery rate of about 16% more nearly eventually than a cell-free extract. This enzyme preparation was uniform in SDS-polyacrylamide electrophoresis. It is DITC about these refining enzymes. Membrane It is made to stick to [a milli gene / bio-search (Milligen/Biosearch) company make], When the amino acid sequence of the amino terminal was determined using Prosequencer 6625 (a milli gene / bio-search company make), the amino acid sequence of the amino terminal of 5 residue shown in the array number 98 of the array table was determined. Since the amino terminal of refining enzymes was started from the 21st alanine residue of the arrangement of the array number 10 of an array table. The amino acid sequence shown in the array number 10 of an array table is the arrangement of a precursor protein, and it was thought that peptide to the 20th phenylalanine residue was removed from the 1st methionine residue after translation. From this result, it was thought that the amino acid sequence of mature-bodies protein was arrangement shown in the amino acid numbers 1-228 during the arrangement shown in the array number 10 of an array table.

[0051]Example 21 Enterobacter aerogenes IFO 12010 High manifestation Enterobacter aerogenes of the enzyme by change of the promoter sequence of an origin new variant acid phosphatase gene IFO 12010 origin variant acid phosphatase. Site-specific mutation was introduced into the promotor arrangement part of the gene to encode with the gene engineering technique, and the gene which encodes the variant acid phosphatase which the enzyme expression amount increased was built. The gene which introduces variation used plasmid pENP170 built in Example 3 of the Japanese-Patent-Application-No. No. 189226 [12 to] specification. This plasmid is Enterobacter aerogenes. The 1.6kbpDNA fragment cut down with the restriction enzyme Sall containing the gene which encodes IFO 12010 origin variant acid phosphatase, and the restriction enzyme KpnI, It is the plasmid DNA connected with pUC19 (made by TAKARA SHUZO CO., LTD.) cut by Sall and Kpnl, and the base sequence of the Sall-KpnI 1.6kbpDNA fragment in pENP170 is arrangement shown in the array number 9 of an array table. The variation introduction to plasmid DNA used the Stratagene quick change site-directed-mutagenesis kit (Quick Change site-directed mutagenesis kit). Oligonucleotide MUT170 for variation introduction compounded using the DNA synthesizer (Applied Biosystem model 394) (array number 99 of an array table), Variation was introduced according to the protocol of Stratagene, using pENP170 as MUT171 (array number 100 of an array table), and a mold. Escherichia coli JM109 (made by TAKARA SHUZO CO., LTD.) was transformed with the conventional method using the obtained plasmid DNA. Plating of this was carried out on L agar medium containing 100 microg/ml ampicillin, and the transformant was obtained. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. Determination of a base sequence Taq DyeDeoxy Terminator Cycle Sequencing Kit (made by an applied blochemical company) is used, and it is the method of Sanger and others. It carried out according to [journal OBU leakage-at-bulb cue biology (J. Mol. Biol.), the 143rd volume,

and the 161st page (1980)]. It does in this way. Enterobacter aerogenes IFO. The variant gene which encodes the variant gene with which the base sequence of -10 field of the presumed promoter sequence located upstream of 12010 origin presumption acid phosphatase varied to the base sequence of the same TATAAT as the lac promotor of Escherichia coli from AAAAAT was built. The plasmid containing this variant gene was named pENP180.

[0052]Escherichia coli 50 ml of L culture media which contain ampicillin 100microg/ml for Escherichia coli JM109/pENP180 which introduced the gene which changed JM109/pENP170 and -10 field of the promoter sequence, And 50 ml of L culture media containing ampicillin 100microg/ml which added IPTG1mM were inoculated, respectively, and it cultivated at 37 ** for 16 hours. Biomasses were collected by centrifugal separation from the culture medium of each bacillus, and the physiological saline washed once. It was made to react at 30 ** for 1 hour, maintaining [dissolve 15 g/dl of pyrophoric acid and inosine, and 8 g/dl in a 100mM acetic acid buffer (pH 4.0), add so that it may become 100 mg/dl by dry cell weight about each biomass at this, and] pH to 4.0. The quantity of generated 5'-inosinic acid was shown in Table 11. High performance chromatography (HPLC) analyzed inosine and 5'-inosinic acid on condition of the following.

Column: Cosmosil 5C18-AR (4.6x150 mm) [Nacalai Tesque, Inc. products] mobile phase: — 5mM potassium phosphate buffer (pH 2.8) / methanol = 95/5 rate-of-flow: — 1.0 ml/min temperature: — room temperature detection: — in UV245nm Escherichia coli JM109/pENP170, although activity was low IPTG additive-free, Even if Escherichia coli JM109/pENP180 did not add IPTG, it showed high activity. Escherichia coli JM109/pENP180 revealed still higher activity by adding IPTG, and it was shown that change of promoterregion is effective. [0053]

[Table 11]

凶 株	IPTG	生成 5'ーイノシン酸(g/dl)
エシェリヒア・コリ	無添加	0.73
IM109/pEMP170	1 mM 添加	3.09
エシェリヒア・コリ	無添加	2.86
JM109/pENP180	1 mM添加	3.37

[0054]Example 22 Enterobacter aerogenes whose compatibility over a nucleoside improved IFO 12010 Enterobacter aerogenes built in construction example 21 of the origin new variant acid phosphatase gene IFO. Site-specific mutation was introduced into the 12010 origin variant acid phosphatase gene with the gene engineering technique, and the gene which encodes the variant acid phosphatase whose compatibility over a nucleoside, especially guanosine improved was produced. The substitution of amino acid residue was introduced combining the substitution of the amino acid residue identified contributing to the improvement in compatibility with a nucleoside based on the spacial configuration analysis of an Escherichia BURATTAE enzyme. The variation introduction to plasmid DNA used the Stratagene quick change site-directed-mutagenesis kit (Quick Change site-directed mutagenesis kit). 20 kinds of oligonucleotides for variation introduction from MUT180 (array number 101 of an array table) to MUT521 (array number 120 of an array table) were compounded using the DNA synthesizer (Applied Biosystem model 394). According to the protocol of Stratagene, variation was introduced as first mold, using MUT180 and MUT181 as pENP170 and an oligonucleotide for variation introduction. Escherichia coli JM109 (made by TAKARA SHUZO CO., LTD.) was transformed with the conventional method using the obtained plasmid DNA. Plating of this was carried out on L agar medium containing 100 microg/ml ampicillin, and the transformant was obtained. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. Determination of a base sequence In accordance with the method (the abovementioned academic journal) of Sanger and others, it carried out using Tag DyeDeoxy Terminator Cycle Sequencing Kit (made by an applied biochemical company). Thus, the 153rd threonine residue (ACC) built the gene which encodes the variant acid phosphatase replaced by serine residue (TCC), and named pENP200 the plasmid containing this variant gene. The operation same as a new mold was repeated for the plasmid which introduced variation, and site-specific mutation was introduced

cumulatively. It checked that prepared a plasmid by an alkali bacteriolysis method, determined a base sequence, and the target base was replaced from the transformant. The variant enzyme gene and mutation site which encode the created variant acid phosphatase were shown in Table 12. The amino acid residue of the mutation site shows the amino acid residue in the amino acid sequence of the maturation protein shown in the array number 10 of the array table.

[0055]Each variant acid phosphatase gene. The included plasmid. introduced Escherichia coli . JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. The biomass was suspended to a 50-ml 100mM phosphoric acid buffer (pH 7.0), ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. Biomasses were collected by centrifugal separation from the culture medium of each bacillus, and the physiological saline washed once. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. Km value to the inosine and guanosine in a phosphotransfer reaction was measured using each cell-free extract.

[0056]Measurement of the phosphate transfer activity to a nucleoside was performed by using inosine and guanosine as a substrate on the following conditions. pH 4.0 was performed by the reaction mixture (1 ml) containing the inosine or the guanosine, 100 micro a mol [/ml] sodium pyrophosphate, the 100 micro mol/ml sodium acetate buffer solution (pH 4.0), and the enzyme of various concentration, and the reaction was performed at 30 ** for 10 minutes. After adding 2N chloride 200mul and suspending a reaction, under [a fixed quantity / which was generated by the phosphotransfer reaction except for precipitate by centrifugal separation / 5'-inosinic acid or 5'guanylic acid]. High performance chromatography (HPLC) analyzed inosine, guanosine, 5'-inosinic acid, and 5'-guanylic acid on the same conditions as Example 21. The concentration of inosine or guanosine is changed on the reaction condition of the above-mentioned presentation, phosphate transfer activity is measured, and it is a Hanes-Woolf plot. It asked for the kinetic constant of the inosine in a phosphotransfer reaction, and guanosine by [a ZABAIO chemical journal (Biochem.J.), the 26th volume, and the 1406th page (1932)]. The result was shown in Table 13 - 16. It became clear that Km value to guanosine falls notably and the compatibility of Km value of the enzyme variant created in the example over guanosine is improving. Km value [as opposed to inosine in four kinds of enzyme variants other than the enzyme variant by which a code is carried out to pENP520] was also falling dramatically.

[0057] [Table 12]

配列の名称	配列番号		ちみ	配 列
MUT170	99	センス	30	5'-CTT ACA GAT GAC <u>TAT AAT</u> GTG ACT AAA AAC
MUT171	100	アンチセンス	30	5'-GTT TTT AGT CAC ATT ATA GTC ATC TGT AAG
MUT180	101	センス	33	5'-TCT ACC GGT TGG GCA TCC GCG CTG GTA CTG GCG
HUT181	102	アンチセンス	33	5'-CGC CAG TAC CAG CGC GGA TGC CCA ACC GGT AGA
MUT300	103	センス	33	5'-TCC GGC CAT ACC TCT TCC GGT TGG GCA TCC GCG
MUT301	104	アンチセンス	33	5'-CGC GGA TGC CCA ACC GGA AGA GGT ATG GCC GGA
MUT310	105	センス	33	5'-GAT GCT GAC CTG GCC GTT GGC GAC GTC GCG AAT
MUT311	106	アンチセンス	33	5'-ATT CGC GAC GTC GCC AAC GGC CAG GTC AGC ATC
MUT320	107	センス	33	5'-CTG ACA AAT ATG ATT CTG GAT GCC GGC GAT CTG
MUT321	108	アンチセンス	33	5'-CAG ATC GCC GGC ATC CAG AAT CAT ATT TGT CAG
MUT330	109	センス	33	5'-GAT GCT GAC CTG GCC ATG GGC GAC GTC GCG AAT
MUT331	110	アンチセンス	33	5'-ATT CGC GAC GTC GCC CAT GGC CAG GTC AGC ATC
MUT340	111	センス	33	5'-CTG ACA AAT ATG ATT CAG GAT GCC GGC GAT CTG
MUT341	112	アンチセンス	33	5'-CAG ATC GCC GGC ATC CTG AAT CAT ATT TGT CAG
MUT400	113	センス	33	5'-TCC GGC CAT ACC TCT GCT GGT TGG GCA TCC GCG
MUT401	114	アンチセンス	33	5'-CGC GGA TGC CCA ACC AGC AGA GGT ATG GCC GGA
MUT500	115	センス	33	5'-TCC GGC CAT ACC TCT GGC GGT TGG GCA TCC GCG
MUT501	116	アンチセンス	33	5'-CGC GGA TGC CCA ACC GCC AGA GGT ATG GCC GGA
MUT510	117	センス	33	5'-GAT GCT GAC CTG GCC GAA GGC GAC GTC GCG AAT
MUT511	118	アンチセンス	33	5'-ATT CGC GAC GTC GCC TTC GGC CAG GTC AGC ATC
MUT520	119	センス	33	5'-GAT GCT GAC CTG GCC AAA GGC GAC GTC GCG AAT
MUT521	120	アンチセンス	33	5'-ATT CGC GAC GTC GCC ITT GGC CAG GTC AGC ATC

[0058] [Table 13]

プラスミド名	変異を導入し	変異導入に用い	一次日 上 アフィーフ 1 正角照上位
ノノハミド石	たプラスミド	大学ライマー	変異点及びアミノ酸置換
pENP180	(ルノノハミ)	16771 V	OST (CMC)
PLIN 100			61L(CTG)→Q(CAG) 63A(GCT)→Q(CAG)
		į	
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
- Pamano	DND190	lmims on lmms os	151I(ATC)→T(ACC)
pENP200	pENP130	MUT180, MUT181	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
DVDAGA	7777		151T(ACC)→S(TCC)
pENP300	pENP200	MUT300, MUT301	61L(CTG)→Q(CAG)
		-	68A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			$133T(ACC) \rightarrow K(AAA)$
			134E(GAG)→D(GAC)
	ļ		151I(ATC)→T(ACC)
			$149T(ACC) \rightarrow S(TCC)$
	,		151T(ACC)→S(TCC)
pENP310	pENP300	MUT310, MUT311	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			$69S(AGC) \rightarrow A(GCC)$
			$70A(GCC) \rightarrow V(GTT)$
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
	1		$151I(ATC) \rightarrow T(ACC)$
			$149T(ACC) \rightarrow S(TCC)$
- END GOO	7117010		151T(ACC)→S(TCC)
pENP320	pENP310	MUT320, MUT321	61L(CTG)→Q(CAG)
		V	63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
		***************************************	67N(AAC)→D(GAC)

[0059] [Table 14]

			$69S(AGC) \rightarrow A(GCC)$
	\		70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)
	1		102E(GAG)→L(CTG)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→S(TCC)
			$151T(ACC) \rightarrow S(TCC)$
pENP330	pENP300	MUT330, MUT331	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→M(ATG)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→S(TCC)
			151T(ACC)→S(TCC)
pBNP340	pENP330	MUT340, MUT341	61L(CTG)→Q(CAG)
			69A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)
			102E(GAG)→Q(CAG)
1			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→S(TCC)
			151T(ACC)→S(TCC)
pENP400	DENP200	MUT400, MUT401	61L(CTG)→Q(CAG)
1			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
	}		69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→A(GCT)
			$151T(ACC) \rightarrow S(TCC)$
pENP410	penp400	MUT310, MUT311	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
	}		64E(GAA)→Λ(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→V(GTT)
L	l		72G(GGC)→D(GAC)

[0060] [Table 15]

		-	
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)-A(GCT)
			$151T(ACC) \rightarrow S(TCC)$
pENP500	pENP200	MUT500, MUT501	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
İ			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→G(GGC)
			151T(ACC)→S(TCC)
pENP510	pENP500	MUT510, MUT511	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→E(GAA)
			72G(GGC)→D(GAC)
1			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			$149T(ACC) \rightarrow G(GGC)$
-mmcaa	THE		151T(ACC)→S(TCC)
pENP520	pENP500	MUT520, MUT521	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→K(AAA)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
	}		134E(GAG)→D(GAC)
	1		149T(ACC)→G(GGC)
			151T(ACC)→S(TCC)

[0061] [Table 16]

	イノシンに対	イノシンを基	グアノシンに	グアノシンを
	する Km 値	質とした場合	対するKm値	基質とした時
	(mM)	の相対活性	(Mm)	の相対活性
pENP180	40	1. 0	40	1. 0
pBNP320	19	1. 9	4. 6	1. 5
pENP340	19	1. 4	5. 1	1. 3
pBNP410	18	1. 0	4. 9	0.70
pENP510	17	0.55	4. 0	0.39
pENP520	4 6	0.63	4. 4	0.21

[0062]Example 23 Enterobacter aerogenes whose compatibility over guanosine improved IFO 12010 the variant acid phosphatase gene of each phosphorylation of the guanosine by an origin new variant acid phosphatase transgenics bacillus. The included plasmid. introduced Escherichia coli . JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. 10 g/dl of pyrophoric acid and 6.6 g/dl of grinding guanosine prepared like Example 1 of JP,12–189226,A are dissolved in a 100mM acetic acid buffer (pH 4.5), It

was made to react at 35 ** for 12 hours, maintaining [to this, add each biomass so that it may become 100 mg/dl by dry cell weight, and] pH to 4.5. The quantity of generated 5'-guanylic acid was shown in Table 17. Escherichia coli each whose bacillus which introduced the enzyme variant as shown in Table 17 is an old stock Productivity improved compared with JM109/pENP180, and generation accumulation of the 5'-guanylic acid was carried out with high yield. [0063]

[Table 17]

菌 株	生成 5'-グアニル酸 (g/dl)
エシェリヒア・コリ	9.90
JM109/pENP180 .	9. 90
エシェリヒア・コリ	10.4
JM109/pENP320	10.4
エシェリヒア・コリ	10.2
JM109/pENP340	10.2
エシェリヒア・コリ	11.1
JN109/pENP410	1
エシェリヒア・コリ	11.0
JM109/pENP510	11.0
エシェリヒア・コリ	10.5
JM109/pENP520	10.0

[0064]

[Effect of the Invention] As explained to details above, according to this invention, a variant nucleoside 5'-phosphoric acid production enzyme whose nucleoside 5'-phosphoric acid productivity improved, and a manufacturing method for the same are provided. According to this invention, the microorganism which holds the recombinant DNA which contains in the manufacturing method of nucleoside 5'-phosphoric acid the gene which encodes the useful aforementioned enzyme variant, and this gene, and this recombinant DNA is provided. It succeeded in the break through of the proteinic new spacial configuration by X ray crystal-structure-analysis art. [0065]

[Layout Table]

<110> Ajinomoto Co., Inc. (Ajinomoto Co., Inc.)

<120>. Variant nucleoside 5'-phosphoric acid production enzyme <130> reference number 1-000804-1 <141> 2000-09-03<150> JP 11-249545<151> 1999-09-03<160> <170> Patentin Ver. 2.0[0066] <210> 1<211> 1225<212> DNA<213> Escherichia blattae<220> <221> CDS< 222> (331)..(1077)<400> 1 ctgcaggcga aaggcaatgt, ggtggccggt gagacggcac, tctacgagat taaggataag, 60 taactatcca ttattacagg, taacagcatt gctcctgagt. gtgatgtcat acctgagcgg. 120 cgcgggggtt ccccgggccg, ctttttttta tggggctgcg. gtgaggagcg ttatctgctg. 180 gccctgtttg tgcaacaaac. gcttttattg tgtaattttt. gtgacgtata tcaggttttt. 240 aagcaccetg tggegeteat, actggeaace tgttgatatt. aagcaacact etteacteae. 300 ggaattaaca egeacagtaa. aggtatacgc atg aaa aaa cgt gtt ctg gca gtt 354 Met Lys Lys Arg Val Leu Ala Val 1 5tgt ttt gcc gca ttg. tto tot tot cag goc. ctg gog ctg gtc gct. acc 402 Cys Phe Ala. Ala Leu Phe Ser Ser. Gln Ala Leu Ala Leu. Val Ala Thr 10 15 20. ggc aac gac act acc. acg aaa ccg gat ctc tac tac ctc aag aac agt 450 Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu Tyr Tyr Leu Lys Asn Ser 25 30 35 40 gaa gcc att aac agc ctg gog ctg ttg cog. coa coa cog gog gtg. ggc 498 Glu Ala Ile. Asn Ser Leu Ala Leu. Leu Pro Pro Pro, Ala Val Gly 45 50 55, too att gog ttt oto, aac gat oag god atg. tat gaa dag ggg ogd, otg 546 Ser Ile Ala. Phe Leu Asn Asp Gln. Ala Met Tyr Glu Gln. Gly Arg Leu 60 65 70. ctg cgc aac acc gaa. cgc ggt aag ctg gog, gog gaa gat goa aac, ctg 594 Leu Arg Asn. Thr Glu Arg Gly Lys, Leu Ala Ala Glu Asp Ala Asn Leu 75 80 85 agc agt ggc ggg gtg gcg aat gct ttc tcc ggc gcg ttt ggt agc ccg 642 Ser Ser. Gly Gly Val Ala Asn. Ala Phe Ser Gly Ala. Phe Gly Ser Pro 90 95. 100 atc acc gaa aaa. gac gcc ccg gcg ctg. cat aaa tta ctg acc. aat atg 690 Ile Thr. Glu Lys Asp Ala Pro. Ala Leu His Lys Leu. Leu Thr Asn Met105 110. 115 120att gag gac gcc. ggg gat ctg gcg acc. cgc agc gcg aaa gat. cac tat 738 lle Glu. Asp Ala Gly Asp Leu. Ala Thr Arg Ser Ala. Lys Asp His Tyr 125. 130 135 atg cgc att. cgt ccg ttc gcg ttt. tat ggg gtc tct acc tgt aat acc 786 Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly Val Ser Thr Cys Asn Thr 140 145 15. O acc gag cag gac aaa. ctg tcc aaa aat ggc. tct tat ccg tcc ggg. cat 834 Thr Glu Gln. Asp Lys Leu Ser Lys. Asn Gly Ser Tyr Pro. Ser Gly His 155 160, 165 acc tot atc ggc. tgg gct act gcg ctg. gtg ctg gca gag atc. aac cct 882 Thr Ser. Ile Gly Trp Ala Thr. Ala Leu Val Leu Ala. Glu Ile Asn Pro

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[Translation done.]

* NOTICES *

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- 1. This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is a figure showing these space position relations with five amino acid residue used as the component of the active site which has phosphatase activity or phosphoryl-group-transfer activity as a distance between Calpha atoms.

[Drawing 2]It is the figure which aligned the amino acid sequence of EB-AP with the amino acid sequence of Morganella MORUGANI, Salmonella typhimurium, and ZAIMO monas MOBIRISU origin acid phosphatase.

[Drawing 3]It is a photograph of the computer graphics (CG) which shows the crystal structure of the bond form model of an EB-AP reaction intermediate analog and inosine.

Drawing #It is a photograph of CG which shows the crystal structure of the hexamer molecule of EB-AP.

Drawing 5]It is a photograph of CG which shows the crystal structure of the subunit of EB-AP.

[Drawing 6]It is a figure showing the active site architecture of EB-AP.

[Drawing 7]It is a figure showing the primer set used for the site-directed-mutagenesis method.

[Drawing 6]It is a figure showing the primer set used for the site-directed-mutagenesis method.

[Drawing 6]It is a figure showing the result of having performed amino acid sequence alignment of EB-AP and Enterobacter aerogenes origin acid phosphatase (EA-AP) by the program BLAST.

[Drawing 10]It is a figure showing the crystallography data (1) of the structure of EB-AP.

[Drawing 11]It is a figure showing the crystallography data (2) of the structure of EB-AP.

[Drawing 13]It is a figure showing the crystallography data (3) of the structure of EB-AP.

[Drawing 14]It is a figure showing the crystallography data (5) of the structure of EB-AP.

[Drawing 15]It is a figure showing the crystallography data (6) of the structure of EB-AP.

[Drawing 14]It is a figure showing the crystallography data (7) of the structure of EB-AP.

[Drawing 14]It is a figure showing the crystallography data (8) of the structure of EB-AP.

[Drawing 14]It is a figure showing the crystallography data (8) of the structure of EB-AP.

[Drawing 19]It is a figure showing the crystallography data (10) of the structure of EB-AP. [Drawing 20]It is a figure showing the crystallography data (11) of the structure of EB-AP.

Drawing 21] It is a figure showing the crystallography data (12) of the structure of EB-AP.

[Drawing 22]It is a figure showing the crystallography data (12) of the structure of EB-AP.

[Drawing 23]It is a figure showing the crystallography data (14) of the structure of EB-AP.

[Drawing 24] It is a figure showing the crystallography data (15) of the structure of EB-AP.

[Drawing 25] It is a figure showing the crystallography data (16) of the structure of EB-AP.

[Drawing 26] It is a figure showing the crystallography data (17) of the structure of EB-AP.

Drawing 27 It is a figure showing the crystallography data (18) of the structure of EB-AP.

[Drawing 28] It is a figure showing the crystallography data (19) of the structure of EB-AP.

[Drawing 29] It is a figure showing the crystallography data (20) of the structure of EB-AP. [Drawing 30] It is a figure showing the crystallography data (21) of the structure of EB-AP.

[Drawing 30]It is a figure showing the crystallography data (21) of the structure of EB-AP.

[Drawing 32]It is a figure showing the crystallography data (23) of the structure of EB-AP.

[Drawing 33] It is a figure showing the crystallography data (24) of the structure of EB-AP.

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Drawing 34]It is a figure showing the crystallography data (25) of the structure of EB-AP. [Drawing 35]It is a figure showing the crystallography data (26) of the structure of EB-AP. [Drawing 36]It is a figure showing the crystallography data (27) of the structure of EB-AP. [Drawing 37]It is a figure showing the crystallography data (28) of the structure of EB-AP. [Drawing 38]It is a figure showing the crystallography data (29) of the structure of EB-AP. [Drawing 39]It is a figure showing the crystallography data (30) of the structure of EB-AP. [Drawing 40]It is a figure showing the crystallography data (31) of the structure of EB-AP. [Drawing 41]It is a figure showing the crystallography data (33) of the structure of EB-AP. [Drawing 43]It is a figure showing the crystallography data (34) of the structure of EB-AP. [Drawing 44]It is a figure showing the crystallography data (35) of the structure of EB-AP. [Drawing 45]It is a figure showing the crystallography data (36) of the structure of EB-AP. [Drawing 46]It is a figure showing the primer set used for the site-directed-mutagenesis method. [Drawing 48]It is a figure showing the primer set used for the site-directed-mutagenesis method. [Drawing 48]It is a figure showing the primer set used for the site-directed-mutagenesis method. [Drawing 48]It is a figure showing the primer set used for the site-directed-mutagenesis method.
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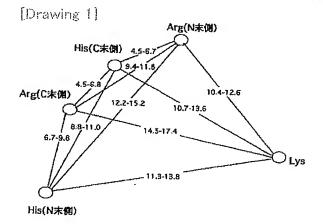
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* NOTICES *

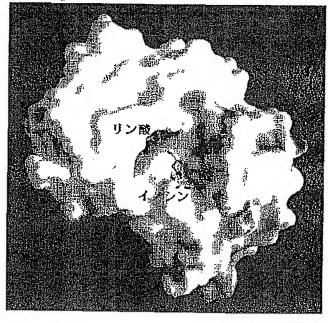
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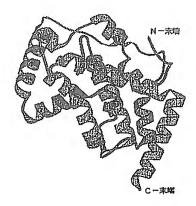
DRAWINGS



[Drawing 3]



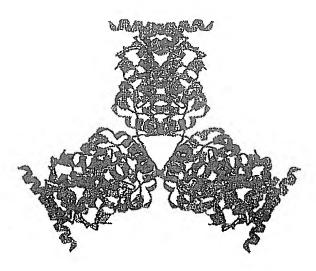
[Drawing 5]



[Drawing 2]

			Ö		0				
	70		40		210				
α3	GKLAAEDANI GKQAQADADI WKQAAEDADV WALATQDADI * **		TCNTTEQDEL TCNTKDQKKI TCRPEDENTL ICTEKDREGL *		GSAVVATLHT GSAAVATLHS GAVEFARLOT	ASGEIAALHG * *			
	QGRLLRNTER KGRMLRNTER KGYALLGSPR STRQLKGSTR	68	ERPEAFYGVS IRPEAFYGTE TREFVLFVHS KREFVDTDQP ***	α12	GENVICGYEM OSHVDAARVV GSAVVATLE OSRVICGYEM OSHVDAARIV GSAAVATLE OSRVICGAEM OSHVDAGRYV GAVEFARLO	HOKT VCGEHW ASHVQAGYIM ASGEIAALHG			
α_2	LPPPBAVGSI AFLNDQAMYE LPPPBEVGSI QFLNDQAMYE LPPPBGNDDP AYRYDKEAYF LAPPPTSGSP LQAHDDQTFN * ***	88			GERVICGYEW OSRVICGYEW GSRVICGAEW	## ## ##			
		α7	NMIEDAGDIA NMIEDAGDIA NMIEDAGOLA NLLTMGGYYA RALRIEYDDI	α11	BX .	* ** ***			ny Kieqsar
	NSEAINSLAL NEGAIDSIKI PEESVNSQFY YHFHSDPLLY	α6	DAPALHKILT NN DSPELYKILT NN DTPETWNALK NI QLPHLANLIK R		POR PER PER	* * *		MM	LLSKEDHPKL NY SAHTPDDLLC KIEQSAR
A DAN TURKUM		α5	SSGGVANAFS GAFGSPITEK AAGGVATAFS GAFGYPITEK. SVENIARIFS PVVGAKINPK HLASVLKDYA CAAGMNLDIA	α10	PSGH TSIGWATALV LAEIN PSGH TSIGWATALV LAEVN PSGH TAYGTLLALV LSEAR	**	α13	akaefaqhok akoefaokso	VREELNDKNN ARKELEKART *
.T.aT.872mcmm	ATPAGNDA :	Q.4	SSGGVANAFS SAGGVATAFS SVENIARIFS HLASVLKDYA		SKNGSYPSGH STNGSYPSGH SKNGSYPSGH	****	Ö	:NPAFQQQLQK AKAEFAQHQK :DPAFQAQLAK AKQEFAQKSQ	:IPAFQKSLAK VREELNDKNN :DADFRRDMEL ARKELEKART *
7. 2. 4. 4. 1. 1. 1. 1.	M.morganii S.typhimurium Z.mobilis		E.blattae M.morganii S.typhimurium Z.mobilis		E.blattae M.morganii S.typhimurium Z.mobilis				S.typhimurium Z.mobilis

[Drawing 4]



[Drawing 7]

```
5'-CA-AAC-CTG-AGC-TTT-GGC-GAT-GTG-GC-3'
 572F(n)
 S72P(as) 3'-GT-TTG-GAC-TCG-AAA-CCG-CTA-CAC-CG-5'
                 ĸ
                    L S F72 G D
 S72Y(B) S'-CA-AAC-CTG-AGC-TAC-GGC-GAT-GTG-GC-3'
 S72Y(as) 3'-GT-TTG-GAC-TCG-ATG-CCG-CTA-CAC-CG-5'
                 K L S Y72 G D Y
 S72W(s) 5'-CA-AAC-CTG-AGC-TGG-GGC-GAT-GTG-GC-3'
 S72W(RE) 3'-GT-TTG-GAC-TCG-ACC-CCG-CTA-CAC-CG-5'
                N L S W72 G D
 S72D(B) 5'-CA-AAC-CTG-AGC-GAC-GGC-GAT-GTG-GC-3'
 S72D(as) 3'-GT-TTG-GAC-TCG-CTG-CCG-CTA-CAC-CG-5'
                N L S D72 G D V
 S72V(s) 5'-CA-AAC-CTG-AGC-GTT-GGC-GAT-GTG-GC-3'
 S72V(as) 3'-GT-TTG-GAC-TCG-CAA-CCG-CTA-CAC-CG-5'
                    L S V72 G D
 S72E(a) 5'-CA-ARC-CTG-AGC-GAA-GGC-GAT-GTG-GC-3'
 S72E(as) 3'-GT-TTG-GAC-TCG-CTT-CCG-CTA-CAC-CG-5'
                H
                    L S E72 G D V
 572M(B) 5'-CA-AAC-CTG-AGC-ATG-GGC-GAT-GTG-GC-3'
 S72H(as) 3'-GT-TTG-GAC-TCG-TAC-CCG-CTA-CAC-CG-5'
                N L S M72 G D
 S72T(a) 5'-CA-AAC-CTG-AGC-ACC-GGC-GAT-GTG-GC-3'
S72T(as) 3'-GT-TTG-GAC-TCG-TGG-CCG-CTA-CAC-CG-5'
                    L S T72 G
                                  D
S72L(B) 5'-CA-AAC-CTG-AGC-CTG-GGC-GAT-GTG-GC-3'
S72L(as) 3'-GT-TTG-GAC-TCG-GAC-CCG-CTA-CAC-CG-5'
                Ħ
                    L S L72 G D
S72R(s) 5'-CA-AAC-CTG-AGC-CGT-GGC-GAT-GTG-GC-3'
S72R(as) 3'-GT-TTG-GAC-TCG-GCA-CCG-CTA-CAC-CG-5'
                N L S R72 G D
S72Q(s) 5'-CA-AAC-CTG-AGC-CAG-GGC-GAT-GTG-GC-3'
S72Q(as) 3'-GT-TTG-GAC-TCG-GTC-CCG-CTA-CAC-CG-5'
                  L S Q72 G D V
                M
         5'-CA-AAC-CTG-AGC-AAA-GGC-GAT-GTG-GC-3'
S72K(as) 3'-GT-TTG-GAC-TCG-TTT-CCG-CTA-CAC-CG-5'
                H
                    L S K72 G
                                  D V
S72P(s)
         5'-CA-AAC-CTG-AGC-CCG-GGC-GAT-GTG-GC-3'
S72P(as) 3'-GT-TTG-GAC-TCG-GGC-CCG-CTA-CAC-CG-5'
                N
                    L S 272 G D
S72X(8)
         5'-CA-AAC-CTG-AGC-GCG-GGC-GAT-GTG-GC-3'
S72A(as) 3'-GT-TTG-GAC-TCG-CGC-CCG-CTA-CAC-CG-5'
                N L S A72 G D
572N(s)
         5'-CA-AAC-CTG-AGC-AAC-GGC-GAT-GTG-GC-3'
S72N(as) 3'-GT-TTG-GAC-TCG-TTG-CCG-CTA-CAC-CG-5'
                N L S N72 G
        5'-CA-AAC-CTG-AGC-GGT-GGC-GAT-GTG-GC-3'
S72G(#)
S72G(as) 3'-GT-TTG-GAC-TCG-CCA-CCG-CTA-CAC-CG-5'
                  L S G72 G D V
               N
S72H(s)
        5'-CA-AAC-CTG-AGC-CAC-GGC-GAT-GTG-GC-3'
S72H(as) 3'-GT-TTG-GAC-TCG-GTG-CCG-CTA-CAC-CG-5'
               N
                  L S H72 G D V
```

[Drawing 8]

```
5'-CG-AAA-CCG-GAT-TGG-TAC-TAC-CTC-AA-3'
L16W(S)
L16W(as)
             3'-GC-TTT-GGC-CTA-ACC-ATG-ATG-GAG-TT-5'
                      K
                           P
                                D
                                     W15 Y
                                               Y
                                                    L
             5'-AT-GCA-AAC-CTG-TGG-AGT-GGC-GAT-GT-3'
S71W(S)
             3'-TA-CGT-TTG-GAC-ACC-TCA-CCG-CTA-CA-5'
S71W(as)
                                                    D
                                     W71 S
                                               6
                      A
                           N
                                L
             5'-AC-CTG-AGC-AGT-TGG-GAT-GTG-GCG-AA-3'
G73W(S)
             3'-TG-GAC-TCG-TCA-ACC-CTA-CAC-CGC-TT-5'
G73W(as)
                      L
                           S
                                S
                                     W73 D
                                               V
                                                    A
             5'-CC-AAT-ATG-ATT-TTT-GAC-GCC-GGG-GA-3'
E104F(S)
E104F(as)
             3'-GG-TTA-TAC-TAA-AAA-CTG-CGG-CCC-CT-5'
                      N
                           M
                                I
                                     F104 D
                                               A
                                                    G
             5'-CC-AAT-ATG-ATT-TGG-GAC-GCC-GGG-GA-3'
E104W(6)
E104W(as)
             3'-GG-TTA-TAC-TAA-ACC-CTG-CGG-CCC-CT-5'
                      N
                           M
                                I
                                     W104 D
                                                    G
[Drawing 9]
          {\tt LALVATGNDTT'IKPDLYYLKNSEAINSLALLPPPPPAVGSIAFLNDQAMYE\underline{Q}GRLLRNTER
EB-AP:
             V GND TTKPDLYYLKN++AI+SLALLPPPP VGSIAFLNDQAMYE+GRLLRNTER
            LVPAGNDATTKPDLYYLKNAQAIDSLALLPPPPEVGSIAFINDQAMYEKGRLLRNTER
EA-AP:
                   [72]
EB-AP:
          GKLAAEDANLSSGGVANAFSGAFGSPITEKDAPALHKLLTNMIEDAGDLATRSAKDHYMR
          GKLAAEDANLS+GGVANAFS AFGSPITEKDAP LHKLLTNMIEDAGDLATRSAK+ YMR
EA-AP:
          GKLAAEDANLSAGGVANAFSSAFGSPITEKDAPQLHKLLTNMIEDAGDLATRSAKEKYMR
                   [70]
          IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
EB-AP:
          IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
EA-AP:
          IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
          QSRVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAKAEFAQHQKK
EB-AP:
          +SRVICGYHWQSDVDAAR+VGSAVVATLHTNPAFQQQLQKAK EFA+ QK
          ESRVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKTQK
EA-AP:
```

[Drawing 10]

ATOM	1	N	GLY		7	35.965	71.208	89.712	1.00	36.57
ATOM	2	ÇA	GLY		7	37.459	71.295	89.574	1.00	31.92
atom	3	С	GLY	A	7	38.160	69.982	89.872	1.00	29.76
ATOM	4	0	GLY	. A	7	39.301	69.858	89.492	1.00	31.81
ATOM	5	N	ASN	A	8	37.485	68.990	90.532	1.00	26.40
ATOM	6	CA	ASN	Α	8	38.284	67.775	90.697	1.00	26.63
ATOM	7	C	ASN	Α	8	38.466	67.018	89.396	1.00	29.21
ATOM	8	0	ASN	A	8	37.736	67.238	88.431	1.00	30.52
ATOM	9	CB	ASN	Α	8	37.677	66.810	91.702	1.00	
ATOM	10	CG	ASN	A	8	37.725	67.396	93.104	1.00	
ATOM	11	OD1	ASN	Α	8	38.751	67.744	93.636	1.00	
ATOM	12	ND2	ASN	A	8	36.545	67.536	93.707		31.60
ATOM	13	N	ASP	Α	9	39,455	66.154	89.463		29.14
ATOM	14	CA	ASP	A	9	39.787	65.216	88,391		30.47
ATOM	15	С	ASP	A	9	40.661	64.081	88.901	1.00	
ATOM	16	0	ASP		9	40.804	63.931	90.110	1.00	
ATOM	17	CB	ASP		9	40.394	65.960	87.195	1.00	
ATOM	18	CG	ASP	A	9	41.802	66.484	87.429		32.66
ATOM	19	OD1			9	42.307	66.333	88.532		35.03
ATOM	20	QD2			9	42.400	67.018	86.493		31.63
ATOM	21	N	THR		10	41.272	63.298	87,998		28.72
ATOM	22	CA	THR		10	42.188	62.228	88.430		28.53
ATOM	23	C	THR		10	43.408	62.655	89.259	1.00	30.10
ATOM	24	Ö	THR		10	43.946	61.944	90.095		29.06
ATOM	25	CB	THR		10	42.692	61.405	87.235		26.05
ATOM	26	OG1	THR		10	43.272	60.172	87.655	1,00	27.75
ATOM	27	CG2	THR		10	43.670	62.174	86.313		23.76
ATOM	28	N	THR		1.1	43.814	63.900	88.996	1.00	30.82
ATOM	29	CA	THR		11	44.932	64.389	89.799		32.79
ATOM	30	C	THR		11	44.605	64.736	91.267		36.32
ATOM	31	0	THR		11	45.435	64.658	92.162	1.00	37.21
ATOM	32	ÇВ	THR		11	45.588	65.591	89.143		30.53
ATOM	33	0G1	THR		11	44.845	66.781	89.359		27.79
ATOM	34	CG2	THR		11	45.899	65.362	87.656		32.16
ATOM	35	N	THR		12	43.317	65.076	91.495		34.81
ATOM	36	CA	THR		12	42.910	65.213	92.900		32.91
ATOM	37	C	THR		12	42.265	63.992	93.549	1.00	33.08
ATOM	38	Õ	THR		12	42.350	63.742	94.736		32.49
ATOM	39	СВ	THR		12	41.963	66.395	93.077	1.00	30.92
ATOM	40	OG1	THR		12	40.719	66.162	92.409		32.04
ATOM	41	CG2	THR		12	42.599	67.667	92.543		29.75
ATOM	42	N		A	13	41.565	63.229	92.703		31.17
ATOM	43	CA		A	13	40.791	62.064	93.174		30.27
ATOM	44	C	LYS		13	40.904	60.812	92.287		31.40
ATOM	45	0	LYS	_	13	39.981	60.348	91.605	1.00	
ATOM	46	СВ	LYS		13	39,294	62.395	93.331	1.00	
ATOM	47	CG	LYS		13	39.001	63.747	93.965	1.00	
ATOM	48	CD	LYS		13	37.536	64.076	94.166		37.86
ATOM	49	CE	LYS		13	36.767	62.909	94.772		47.28
ATOM	50	NZ	LYS		13	35.340	63.270	94.947	1.00	
ATOM	51	N	PRO		14	42.138	60.283	92.279	1.00	
ATOM	52	CA	PRO		14	42.516	59.249	91.290	1.00	
ATOM	53	C	PRO		14	41.823	57.907	91.290		
ATOM	54	ő	PRO		14	41.961	56.989	90.668	1.00	
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[Drawing [1]

ATOM	55	CB	PRO	A	14	44.035	59.145	91.468	1.00 34.46
ATOM	56	CG	PRO	A	14	44,283	59.564	92.920	1.00 33.02
MOTA	57	CD	PRO	A	14	43.225	60.638	93,181	1.00 34.46
MOTA	58	N	ASP	Α	15	41.046	57.815	92.513	1.00 29.27
ATOM	59	CA	ASP	A	15	40.204	56.655	92.809	1.00 28.89
ATOM	60	С	ASP	A	15	38.810	56.684	92.146	1.00 21.76
ATOM	61	0	ASP	A	15	38.078	55,706	92.030	1.00 20.59
ATOM	62	CB	ASP	A	15	40.125	56.599	94.368	1.00 37.60
ATOM	63	CG	ASP	A	15	39.589	57.903	95.080	1.00 45.11
ATOM	64	OD1	ASP	A	15	40.062	59.044	94.817	1.00 45.67
ATOM	65	OD2	ASP	A	15	38.687	57.751	95.922	1.00 49.07
ATOM	66	N	LEU	A	16	38.495	57.910	91.726	1.00 20.49
MOTA	67	CA	LEU	A	16	37,182	58.179	91.135	1.00 23.90
ATOM	68	С	LEU	A	16	37.156	58.814	89.727	1.00 22.23
ATOM	69	O	LEU		16	36.109	59.011	89.134	1.00 23.21
ATOM	70	CB	LEU		16	36.354	59.099	92.029	1.00 23.35
ATOM	71	CG	LEU		16	35.814	58.432	93,297	1.00 25.48
ATOM	72	CD1	LEU		16	34.876	57.253	93.075	1.00 24.05
ATOM	73	CD2	LEU		16	35.092	59.477	94.104	1.00 25.22
ATOM	74	N	TYR		17	38.343	59.175	89.273	1.00 20.96
ATOM	75	CA	TYR		17	38.555	59.605	87.889	1.00 22.04
ATOM	76	c	TYR		17	39.780	58.903	87.334	1,00 22.80
ATOM	77	ō	TYR		17	40.790	58.799	88.021	1.00 23.48
ATOM	78	CB	TYR		17	38.856	61.095	87.711	1.00 18.01
ATOM	79	CG	TYR		17	37.928	62,099	88.371	1.00 24.78
ATOM	80	CD1	TYR		17	37.129	62.916	87,542	1.00 22.78
ATOM	81	CD2	TYR		17	37.905	62.248	89.781	1.00 23.58
ATOM	82	CE1	TYR		17	36.317	63.919	88.113	1.00 26.51
MOTA	83	CE2	TYR		17	37.090	63.240	90.349	1.00 22.88
MOTA	84	CZ	TYR		17	36.303	64.059	89.517	1.00 24.63
ATOM	85	ОH	TYR		17	35.482	65.023	90.066	1.00 22.92
ATOM	86	N	TYR		18	39.670	58.482	86.053	1.00 26.17
ATOM	87	CA	TYR		18	40.838	58.209	85.191	1,00 21.13
ATOM	88	C	TYR		18	41.332	59.414	84.464	1.00 19.92
ATOM	89	Q.	TYR		18	42.490	59.511	84.083	1.00 22.64
ATOM	90	СВ	TYR		18	40.563	57.195	84.080	1.00 17.53
ATOM	91	CG	TYR		18	40.312	55.826	84.610	1.00 16.91
MOTA	92	CD1	TYR		18	41.425	55.028	84.916	1.00 19.86
ATOM	93	CD2	TYR		18	38.985	55.372	84.771	1.00 16.65
ATOM	94	CE1	TYR		18	41.218	53.725	85.383	1.00 18.64
ATOM	95	CE2	TYR		18	38.765	54.053	85.213	1.00 17.52
ATOM	96	CZ	TYR		18	39.892	53.262	85.515	1.00 21.18
ATOM	97	OH	TYR		18	39.734	51,974	85.977	1.00 26.15
ATOM	98	N	LEU		19	40.412	60.336	84.236	1.00 21.49
ATOM	99	CA	LEU		19	40.788	61.462	83,366	1.00 22.71
MOTA	100	C	LEU		19	41.094	62.812	84.021	1.00 25.01
ATOM	101	o	LEU		19	40.771	63.125	85.159	1.00 25.24
ATOM	102	CB	LEU		19	39.708	61.669	82.290	1.00 21.68
ATOM	103	CG	LEU		19	39.301	60.442	81.432	1.00 22.88
ATOM	104		LEU		19	40.430	59.842	80.583	1.00 20.39
MOTA	105	CD2	LEU		19	38.078	60.812	80.608	1.00 18.83
ATOM	106	N	LYS		20	41.736	63.667	83,246	1.00 26.44
ATOM	107	ÇA	LYS		20	41.947	65.032	83.717	1.00 26.77
ATOM	108	C	LYS		20	40.935	66.034	83,292	1.00 26.42
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[Drawing 12]

ATOM	109	0	LYS	A	20	40.182	65.870	82.341	1.00	29.05
ATOM	110	CB	LYS	A	20	43.239	65.608	83.187	1.00	30.61
ATOM	111	CG	LYS	A	20	44.400	64.791	83.648		32,90
ATOM	112	CD	LYS	A	20	45.633	65.326	82.963	1.00	39.72
ATOM	113	CE	LYS	A	20	46.698	64.259	83.113	1.00	50.27
ATOM	114	NZ	LY5	A	20	46.148	62.977	82.610	1.00	62.00
ATOM	115	N	ASN	A	21	41.050	67.184	83.943	1.00	24.09
ATOM	116	CA	ASN	A	21	40.154	68.246	83.530	1.00	23.98
ATOM	117	С	ASN	A	21	40.177	68.539	82.032	1.00	25.08
ATOM	118	0	ASN	A	21	39.134	68.722	81.427	1.00	25.36
ATOM	119	CB	ASN	A	21	40.310	69.512	84.371	1.00	23.81
ATOM	120	CG	ASN	A	21	39.601	69.311	85.697	1.00	26.97
ATOM	121	OD1	ASN	A	21	38.392	69.175	85.836	1.00	26.36
ATOM	122	ND2	ASN	A	21	40.403	69.303	86.744	1.00	32.00
ATOM	123	N	SER	A	22	41.378	68.486	81.450	1.00	23.72
ATOM	124	CA	SER	A.	22	41.592	68.804	80.008	1.00	25.53
MOTA	125	С	SER	A	22	40.992	67.752	79.068	1.00	25.77
ATOM	126	0	SER	A	22	40.524	68.007	77.966	1.00	27.65
ATOM	127	CB	SER	A	22	43.079	68.868	79.699	1.00	23.34
ATOM	128	OG	SER	A	22	43.719	67.716	80.303	1.00	33.30
ATOM	129	N	GLU	A	23	40.957	66.529	79.624	1.00	22.35
ATOM	130	CA	GLU	A	23	40.320	65.466	78.899		21.87
ATOM	131	C	GLU	A	23	38.811	65.375	78.974	1.00	23.18
ATOM	132	0	GLU	A	23	38.197	64.451	78.471	1.00	25.83
MOTA	133	CB	GLU	A	23	40.923	64.165	79.337	1.00	22.13
ATOM	134	CG	GLU	Α	23	42.451	64.215	79.214	1.00	26.78
ATOM	135	CD	GLU	A	23	43.021	62.908	79.718	1.00	30.40
ATOM	136	OE1	GLU	A	23	42.946	62.648	80.900	1.00	31.10
MOTA	137	OE2	GLU	A	23	43.544	62.118	78.957	1.00	32.35
ATOM	138	N	ALA	A	24	38.196	66.359	79.610	1.00	21,49
ATOM	139	CA	ALA	A	24	36.751	66.165	79.738	1.00	22.48
ATOM	140	C	ALA	A	24	35.973	66.420	78.438	1.00	22.81
ATOM	141	0	ALA	A	24	36.325	67.333	77.704	1.00	23.36
ATOM	142	CB	ALA	A	24	36.188	67.183	80.734	1.00	21.43
ATOM	143	N	ILE	A	25	34.859	65.694	78.228	1.00	23.46
ATOM	144	CA	ILE	A	25	33.845	66.149	77,243	1.00	23,60
ATOM	145	C	ILE	A	25	33.312	67.535	77.530	1.00	24.71
ATOM	146	0	ILE	A	25	32.788	67.809	78.603	1.00	25.03
ATOM	147	CB	ILE	A	25	32,684	65.160	77.096	1.00	20.26
ATOM	148	CGl	ILE	A	25	33.237	63.749	76.838	1.00	23.14
ATOM	149	CG2	ILE	A	25	31.739	65.555	75.954	1.00	21.26
ATOM	150	CD1	ITE	A	25	34.298	63.551	75.722	1.00	16.94
ATOM	151	N	ASN	A	26	33,485	68.431	76.562	1.00	22.50
ATOM	152	CA	ASN	A	26	32.797	69.706	76.751	1.00	22.04
ATOM	153	С	ASN	A	26	31.295	69.680	76.533	1.00	22.52
ATOM	154	0	ASN	A	26	30.731	70.042	75.509	1.00	22.34
ATOM	155	CB	ASN	A	26	33.474	70.744	75.900	1.00	20.15
ATOM	156	CG	ASN	A	26	32.982	72.133	76.217	1.00	24.94
ATOM	157	OD1	ASN	A	26	31.923	72.459	76.732	1.00	29.46
ATOM	158	ND2	ASN	A	26	33.827	73.032	75.809	1.00	25.76
ATOM	159	N	SER	A	27	30.627	69.289	77.622	1.00	19.77
ATOM	160	CA	SER	A	27	29.166	69.168	77.549	1.00	18.88
ATOM	161	C	SER	A	27	28.412	70.423	77.177	1.00	18.74
ATOM	162	0	SER	A	27	27.390	70.393	76.517	1.00	21.73

[Drawing 13]

MOTA	163	CB	SER	A	27	28.606	68.619	78.870	1.00	19.35
ATOM	164	OG	SER	A	27	28.967	69.518	79.940	1.00	19.36
ATOM	165	N	LEU		28	28.961	71.564	77.588		18.08
ATOM	166	CA	LEU		28	28.271	72.815	77.262		20.15
ATOM	167	C	LEU		28	28.283	73.062	75.761	1.00	23.12
ATOM	168	0	LEU		28	27.303	73.485	75.165	1.00	22,25
ATOM	169	CB	LEU		28	28.990	74.042	77.798	1.00	17.00
ATOM	170	CG	LEU		28	28.159	75.188	78.376		18.01
ATOM	171	CD1	LEU		28	26.847	75.547	77.733		14.28
MOTA	172	CD2	LEU		28	29.053	76.394	78.592		16.45
ATOM	173	N	ALA		29	29.478	72.767	75.193		23.87
ATOM	174	CA	ALA		29	29.598	72.827	73.707		22.62
ATOM	175	С	ALA		29	28.773	71.847	72.837		20.86
MOTA	176	O	ALA		29	28.192	72.239	71.830		25.89
ATOM	177	СВ	ALA		29	31.065	72.692	73.326		20.24
ATOM	178	N	LEU		30	28.733	70.580	73.267		15.78
ATOM	179	CA	LEU		30	28.079	69.497	72.519		18.05
MOTA	180	C	LEU		30	26.557	69.416	72.559		22.38
MOTA	181	0	LEU		30	25.845	69.251	71.566		23.63
ATOM	182	CB	LEU		30	28.732	68.194	72.977		16.47
MOTA	183	CG	LEU		30	28.234	66.887	72.360		17.59
atom	184	CDI	LEU		30	28.812	65.706	73,120		12.95
MOTA	185	CD2	LEU		30	28,456	66.775	70.850		13.89
ATOM	186	N	LEU		31	26.075	69.533	73.812		22.39
ATOM	187	CA	LEU		31	24.633	69.430	74.049		18.84
MOTA	188	C	LEU		31	23.817	70.624	73.538	1.00	16.30
ATOM	189	0	LEU		31	24.260	71.763	73.576		19.93
ATOM	190	CB	LEU		31	24.381	69.199	75.556		16.94
MOTA	191	CG	LEU		31	24.923	67.873	76.095		17.95
ATOM	192	CD1	LEU		31	24.177	66.669	75.553		11.60
ATOM	193	CD2	LEU		31	24.823	67.878	77.628		18.77
ATOM	194	N	PRO		32	22.581	70.333	73.105		14.80
ATOM	195	CA	PRO		32	21.589	71.404	72.910		18.31
ATOM	196	C	PRO		32	21.228	72.028	74.278		22.24
ATOM	197	0	PRO		32	21.453	71.442	75.327		22.27
ATOM	198	CB	PRO		32	20.402	70.621	72.348		15.94
ATOM	199	CG	PRO		32	20.545	69.184	72.847		16.93
MOTA	200	CD	PRO		32	22.038	68.972	72.954		15.56
ATOM	201	N	PRO		33	20.657	73.249	74.287		23.41
ATOM	202	CA	PRO		33	20.190	73.780	75.586		20.34
ATOM	203	C	PRO		33	19.059	72.945	76.084		19.93
ATOM	204	0	PRO		33	18,409	72.292	75.285		18.67
ATOM	205	CB	PRO		33	19.659	75.158	75.224	1.00	18.52
ATOM	206	CG	PRO	A	33	20.267	75.499	73.877		21.71
ATOM	207	CD	PRO		33	20.406	74.146	73.177		21.12
ATOM	208	N	PRO		34	18.785	72.950	77.411		19.36
ATOM	209	CA	PRO		34	17.645	72.138	77.863		13,70
MOTA	210	С	PRO		34	16.348	72.759	77.351		11.77
MOTA	211	0	PRO		34	16.280	73.937	77.090		14.58
MOTA	212	CB	PRO		34	17.760	72.358	79.389		13.66
ATOM	213	CG	PRO		34	18.471	73.698	79.571		14.81
ATOM	214	CD	PRO		34	19.499	73.679	78.464		16.49
ATOM	215	N	PRO		35	15.257	72.007	77.284		12.52
ATOM	216	CA	PRO	A	35	14.011	72.710	76.973	1.00	13.71

(Drawing 14)

MOTA	217	С	PRO	A	35	13,665	73.842	77.945	1.00	20.26
ATOM	218	0	PRO	A	35	13.728	73.715	79.159		20.52
MOTA	219	CB	PRO	A	35	12.997	71.579	76.991	1.00	11.74
ATOM	220	CG	PRO	A	35	13.723	70.243	77.051	1.00	12.04
ATOM	221	CD	PRO	A	35	15.140	70.581	77.482	1.00	11.57
ATOM	222	N	ALA	A	36	13.311	74.962	77.356	1.00	19.25
ATOM	223	CA	ALA	A	36	12.919	76.136	78.122	1.00	18.78
ATOM	224	C	ALA	A	36	11.457	76.120	78.497	1.00	18.25
ATOM	225	0	ALA	A	36	10.582	75.579	77.847	1.00	18.88
ATOM	226	CB	ALA	A	36	13.152	77.414	77.304	1.00	17.95
ATOM	227	N	VAL	A	37	11.182	76.753	79,609	1.00	18.03
ATOM	228	CA	VAL	A	37	9.803	77.005	79.965	1.00	16.78
MOTA	229	C	VAL	A	37	9.135	77.993	78.998	1.00	17.18
ATOM	230	0	VAL	A	37	9.640	79.048	78.650	1.00	19.89
ATOM	231	CB	VAL	A	37	9.740	77.500	81.436	1.00	18.33
ATOM	232	CG1	VAL	A	37	10.381	76.501	82.418	1.00	13.83
ATOM	233	CG2	VAL	A	37	8.300	77.825	81.832	1.00	14.21
ATOM	234	N	GLY	A	38	7.952	77.616	78.561	1.00	18.34
ATOM	235	CA	GLY	A	38	7.422	78,249	77.343	1.00	22.06
ATOM	236	C	GLY	A	38	7.538	77.398	76.043	1.00	21.25
ATOM	237	0	GLY	A	38	6.851	77.623	75.068	1.00	22.09
ATOM	238	N	SER	A	39	8.422	76,401	76.060	1.00	21.73
ATOM	239	CA	SER	A	39	8.520	75,487	74.905	1.00	20.30
ATOM	240	¢	SER	A	39	7.604	74.277	74.964	1.00	21.10
ATOM	241	0	SER	A	39	7.217	73.736	76.002	1.00	19.55
ATOM	242	CB	SER	A	39	9.946	74.998	74.748	1.00	15.45
ATOM	243	OG	SER	Α	39	10,197	73,967	75.704	1.00	15.38
ATOM	244	N	ILE	A	40	7.287	73.796	73.772	1.00	17,17
ATOM	245	CA	ILE	A	40	6.618	72.485	73.702	1.00	14.71
ATOM	246	C	ILE	A	40	7.475	71.311	74.225	1.00	10.81
ATOM	247	0	ILE	A	40	6.998	70.315	74.782	1.00	15.23
ATOM	248	CB	ILE	A	40	6.102	72,235	72.219	1.00	15.78
ATOM	249	CG1	ILE	A	40	5.162	73.368	71.791	1.00	15.41
ATOM	250	CG2	ILE	A	40	5.406	70.863	72.091	1.00	14.54
ATOM	251	CD1	ILE	A	40	4.812	73.332	70.307	1.00	18.26
ATOM	252	N	ALA	A	41	8.790	71.443	74.040	1.00	10.69
ATOM	253	CA	ALA	A	41	9.633	70.373	74.530	1.00	13.79
ATOM	254	C	ALA	A	41	9.566	70.300	76.091	1.00	15.36
ATOM	255	0	ALA	A	41	9.369	69.245	76.683	1.00	20.02
ATOM	256	CB	ALA	A	41	11.046	70.610	74.065	1.00	11.61
ATOM	257	N	PHE	A	42	9.547	71.495	76.702	1.00	16.94
ATOM	258	CA	PHE	A	42	9.200	71.480	78.151	1.00	15.75
ATOM	259	C	BHE	A	42	7.818	70.970	78.533	1.00	16.07
ATOM	260	0	PHE	A	42	7.652	70.182	79.448	1.00	19.72
ATOM	261	CB	PHE	A	42	9.513	72.819	78.819	1.00	17.93
ATOM	262	CG	PHE	A	42	9.380	72.700	80.338	1.00	20.96
ATOM	263	CD1	PHE	A	42	10.297	71.904	81.056	1.00	19.46
ATOM	264	CD2	PHE	A	42	8.324	73.370	80.997	1.00	20.99
ATOM	265	CEl	PHE	A	42	10.148	71.763	82.450	1.00	17.30
MOTA	266	CE2	PHE	A	42	8.190	73,248	82.402	1.00	19.79
ATOM	267	CZ	PHE	A	42	9.111	72.443	83.100	1.00	
MOTA	268	N	LEU	A.	43	6.790	71.375	77.765	1.00	19.00
ATOM	269	CA	LEU	A	43	5.507	70.643	77.917	1.00	19.22
ATOM	270	C	LEU	A	43	5.573	69.103	77.945	1.00	19.39

[Drawing 15]

MOTA	271	0	LEU		43	4.957	68.410	78.749	1.00 17.69
MOTA	272	CB	LEU		43	4.472	71.003	76.826	1.00 21.60
MOTA	273	CG	TEA		43	3.213	71.850	77.034	1.00 24.67
MOTA	274	CD1	LEU		43	2.597	71.800	78.433	1.00 17.68
MOTA	275	CD2	LEU		43	2,172	71.549	75.953	1.00 21.72
MOTA	276	N	ASN		44	6.392	68.588	77.023	1.00 19.47
ATOM	277	CA	ASN	A	44	6.653	67.176	77.076	1.00 19.82
MOTA	278	С	ASN	A	44	7.419	66,619	78.312	1.00 17.44
ATOM .	279	O	asn	A	44	7.018	65.604	78.855	1.00 15.52
ATOM	280	CB	asn	A	44	7.259	66.847	75.747	1.00 19.07
ATOM	281	CG	ASN	A	44	7.491	65.366	75.643	1.00 22.97
ATOM	282	QD1	asn	A	44	8.605	64.906	75.468	1.00 30.35
ATOM	283	ND2	ASN	A	44	6.444	64.588	75.862	1.00 22.13
ATOM	284	N	ASP	A	45	8.482	67.324	78.726	1.00 19.71
MOTA	285	CA	ASP	A	45	9.175	67.050	80.020	1.00 19.87
ATOM	286	С	ASP	A	45	8.192	66.977	81.213	1.00 19.30
MOTA	287	0	ASP	A	45	8.103	66.009	81.956	1.00 21.00
ATOM	288	CB	ASP	A	45	10.225	68.119	80.273	1.00 13.57
ATOM	289	CG	ASP	A	45	11.563	67.769	79,706	1.00 12.64
ATOM	290	OD1	ASP	A	45	12.408	68.656	79.625	1.00 15.68
ATOM	291	OD2	ASP	A	45	11.823	66.611	79.414	1.00 14.57
ATOM	292	N	GLN	A	46	7.347	68.007	81.299	1.00 19.62
ATOM	293	CA	GLN	A	46	6.199	67.904	82,220	1.00 19.44
ATOM	294	C	GLN	A	46	5.259	66.702	82.166	1.00 22.23
ATOM	295	0	GLN	A	46	4.960	66.057	83.175	1.00 21.67
ATOM	296	CB	GLN	Α	46	5.353	69.153	82.218	1.00 16.35
ATOM	297	CG	GLN	A	46	6.282	70.333	82.395	1.00 18.35
ATOM	298	CD	GLN	A	46	5.398	71.519	82.591	1.00 26.07
ATOM	299	QE1	GLN	A	46	5.334	72.143	83.629	1.00 31.83
ATOM	300	NE2	GLN	A	46	4.622	71.823	81,591	1.00 22.82
ATOM	301	N	ALA		47	4.838	66.364	80.935	1.00 19.12
ATOM	302	ÇA	ALA	A	47	3.979	65.187	80.813	1.00 17.83
ATOM	303	C	ALA		47	4.661	63.871	81.172	1.00 15.90
ATOM	304	0	ALA	A	47	4.065	62.940	81.701	1.00 18.55
ATOM	305	CB	ALA	A	47	3.441	65.066	79.367	1.00 17.11
ATOM	306	N	MET		48	5.970	63.818	80.841	1.00 18.16
ATOM	307	CA	MET	A	48	6.799	62.644	81,235	1.00 19.52
ATOM	308	Ç	MET	A	48	7.012	62.460	82.765	1.00 21.38
ATOM	309	0	MET	A	48	6.996	61.358	83.316	1.00 20.83
ATOM	310	CB	MET	A	48	8.173	62.667	80.539	1.00 21.42
ATOM	311	CG	MET	A	48	8.150	62.603	78.984	1.00 29.81
ATOM	312	SD	MET	A	48	7.330	61.126	78.308	1.00 36.20
ATOM	313	CE	MET		48	5.582	61.633	78.280	1.00 33.60
ATOM	314	N	TYR	A	49	7.139	63.655	83.414	1.00 21.32
ATOM	315	CA	TYR		49	7.066	63.807	84.885	1.00 21.30
ATOM	316	С	TYR		49	5.773	63.244	85.515	1.00 22.58
ATOM	317	0	TYR		49	5.797	62.383	86.390	1.00 24.04
ATOM	318	ÇB	TYR		49	7.304	65.282	85.217	1.00 20.61
ATOM	319	CG	TYR		49	7.034	65.494	86.692	1.00 23.57
ATOM	320	CD1	TYR		49	5.755	65.931	87.109	1.00 23.57
ATOM	321		TYR		49	8.080	65.194	87.574	1.00 21.83
ATOM	322	CE1	TYR		49	5.524	66.097	88.481	1.00 26.09
ATOM	323	CE2	TYR		49	7.844	65,349	88.943	1.00 23,18
ATOM	324	CZ	TYR		49	6.591	65.842	89.377	1.00 26.31
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[Drawing 16]

ATOM	325	OH	TYF	A	49	6.444	66.124	90.726	1.00 29.46
MOTA	326	N	GLU	A	50	4.639	63.731	84.994	1.00 22.09
MOTA	327	CA	GLU	A.	50	3.336	63.234	85.472	1.00 21.48
ATOM	328	Ç	GLU	A	50	3.052	61.776	85.230	1.00 23.20
ATOM	329	0	GLU	A	50	2.548	61.050	86.081	1.00 24.23
MOTA	330	CB	GLU	A	50	2.190	64.023	84.862	1.00 21.88
MOTA	331	CG	GLU	A	50	2.304	65.537	84.986	1.00 21.13
ATOM	332	CD	GLU	Α	50	2.054	65.976	86.417	1.00 25,41
MOTA	333	OE1	GLU	A	50	1.887	65.138	87.287	1.00 24.65
ATOM	334	OE2	GLU	Α	50	2.004	67.162	86.679	1.00 25.05
ATOM	335	N	GLN	A.	51	3,479	61.343	84.032	1.00 23.02
ATOM	336	CA	GLN	A	51	3.427	59.907	83.812	1.00 24.72
ATOM	337	С	GLN		51	4.275	59.006	84.728	1.00 26.23
ATOM	338	0	GLN		51	3.804	57.996	85.253	1.00 25.10
ATOM	339	CB	GLN		51	3.680	59.545	82.355	1.00 24.41
ATOM	340	ÇĢ	GLN		51	3.461	58.028	82.141	1.00 38.05
ATOM	341	CD	GLN		51	2.115	57.435	82.657	1.00 53.15
ATOM	342	QE1	GLN		51	1.093	58.076	82.867	1.00 61.03
ATOM	343	NE2	GLN		51	2.098	56.123	82.834	1.00 55.24
ATOM	344	N	GLY		52	5.556	59.414	84.922	1.00 26.76
ATOM	345	CA	GLY		52	6.400	58.689	85.876	1.00 26.91
ATOM	346	¢	GLY		52	5,793	58.681	87.286	1.00 25.60
ATOM	347	ŏ	GLY		52	5.666	57.699	87.997	1.00 24.37
ATOM	348	N	ARG		53	5.321	59.874	87.621	1.00 28.09
ATOM	349	CA	ARG		53	4.527	60.032	88.834	1.00 20.09
ATOM	350	c	ARG		53	3.384	59.049	89.067	1.00 32.01
ATOM	351	ō	ARG		53	3.284	58.437	90.115	1.00 34.69
ATOM	352	CB	ARG		53	4.128	61.494	88.965	1.00 30.41
ATOM	353	CG	ARG		53	3.857	61.919	90.389	1.00 29,15
ATOM	354	CD	ARG		53	3.519	63.393	90.461	1.00 29.38
ATOM	355	NE	ARG		53	2.385	63.740	89.609	1.00 29.38
ATOM	356	CZ	ARG		53	1.088	63.593		
ATOM	357	NH1	ARG		53	0.187	64.144	89.886 89.125	1.00 32.29
ATOM	358	NH2	ARG		53	0.661	62.938		1.00 32.06
ATOM	359	N	LEU		54	2.575	58.839	90.931	1.00 32.66
ATOM	360	CA	LEU		54			88.033	1.00 32.47
ATOM	361	CA				1.588	57.735	88.076	1.00 31.67
ATOM	362		LEU		54	2.114	56.273	88.119	1.00 33,30
		0	LEU		54	1.452	55.329	88.568	1.00 35.90
ATOM ATOM	363 364	CB	LEU		54	0.603	57.880	86.901	1.00 33.52
		CG	LEU		54	-0.599	58.822	87.055	1.00 32.85
ATOM	365	CD1	LEU		54	~1.298	59.020	85.709	1.00 30.53
ATOM	366	CD2	LEU		54	-0.286	60.130	87.777	1.00 36.56
ATOM	367 368	N	LEU		55 ==	3.374	56.117	87.657	1.00 31.95
ATOM ATOM		CA	LEU		55	4.016	54.784	87.735	1.00 32.70
	369	C	LEU	A	55	4.577	54.392	89.091	1.00 33.98
ATOM	370	0	LEU		55	4.842	53.224	89.386	1.00 32.73
MOTA	371	CB	LEU		55	5.194	54.646	86.790	1.00 31.04
ATOM	372	CG CD1	LEU		55	4.832	54.514	85,343	1.00 28.60
ATOM	373		LEU		55	3.960	53.287	85.101	1.00 27.88
ATOM	374		LEU		55	6.118	54.471	84.539	1.00 28.33
ATOM	375	N	ARG		56	4.732	55.458	89.911	1.00 36.20
ATOM	376	CV	ARG		56	5.257	55.308	91.281	1.00 37.04
ATOM	377	C	ARG		56	4.616	54.240	92.164	1.00 40.28
MOTA	378	0	ARG	A	56	5.260	53.518	92.907	1.00 41.22

[Drawing 17]

ATOM	379	CB	ARG	A	56	5.249	56.643	91.993		32.58
ATOM	380	CG	ARG	A	56	6.368	57.502	91.476	1.00	
ATOM	381	CD	ARG	Α	56	6.142	58.874	92.049		21.74
ATOM	382	NE	ARG	A	56	7.073	59.804	91.447		23.56
ATOM	383	CZ	ARG	A	56	7.062	61.074	91.750		25.56
ATOM	384	NHl	ARG	A	56	6.401	61.444	92.790	1.00	30.94
ATOM	385	NH2	ARG	A	56	7.688	61.979	91.035	1.00	22.33
ATOM	386	N	ASN	A	57	3.306	54.120	91,997	1.00	44.42
ATOM	387	CA	AŞN	A	57	2.602	53.027	92.680	1.00	48.62
ATOM	388	С	ASN	A	57	2,786	51.585	92.169	1.00	47.46
ATOM	389	0	ASN	A	57	2,316	50.630	92.759	1.00	51.15
ATOM	390	CB	ASN	A	57	1.124	53.435	92.726		59.30
ATOM	391	CG	ASN		57	0.389	53.137	94.049	1.00	68.98
ATOM	392	QD1	ASN	A	57	-0.829	53.335	94.164	1.00	75.09
ATOM	393	ND2	ASN		57	1.140	52.692	95.058	1.00	71.68
ATOM	394	N	THR		58	3.461	51.442	91.036	1.00	42.68
ATOM	395	CA	THR		58	3.555	50.086	90.475	1.00	36.64
ATOM	396	C	THR		58	4.821	49.31B	90.871	1.00	33.64
ATOM	397	0	THR		58	5.721	49.876	91.477	1,00	31.69
ATOM	398	СВ	THR		58	3.492	50.189	88.948	1.00	36.81
ATOM	399	og1	THR		58	4.774	50.576	88.447	1.00	37.64
ATOM	400	CG2	THR		58	2.432	51.203	88.507	1.00	35,36
ATOM	401	Ŋ	GLU		59	4.937	48.068	90.409	1.00	33.08
ATOM	402	CA	GLU		59	6.238	47.410	90.581	1.00	34.80
ATOM	403	C	GLU		59	7.487	48.104	89.944	1.00	33.45
ATOM	404	0	GLU		59	8.607	48.153	90.463	1.00	34.28
ATOM	405	СВ	GLU		59	6.067	45.933	90.191	1.00	43.43
ATOM	406	ĊG	GLU		59	7.242	45.007	90.614	1.00	59.74
ATOM	407	CD	GLU		59	7.519	44.933	92.159	1.00	69.61
ATOM	408	OEL	GLU		59	6.582	45.064	92.960	1.00	74.78
ATOM	409	OE2	GLU		59	8.686	44.751	92,589	1.00	74.97
ATOM	410	И	ARG		60	7.229	48.734	88.768	1.00	27.61
ATOM	411	CA	ARG		60	8.251	49.599	88.158	1.00	25.02
ATOM	412	C	ARG		60	8.614	50.851	88.958	1.00	22.94
ATOM	413	ő	ARG		60	9.772	51.257	89.002	1.00	24.63
MOTA	414	CB	ARG		60	7.874	49,966	86.690	1.00	26.16
ATOM	415	ĊĠ	ARG		60	8.877	50.860	85.900	1.00	24.47
ATOM	416	CD	ARG		60	10.268	50.249	85.758	1.00	23.96
ATOM	417	NE	ARG		60	11.285	51,161	85.217	1.00	25.64
ATOM	418	CZ	ARG		60	12.214	51.778	85.945	1.00	24.77
MOTA	419		ARG		60	12.159	51.805	87.261	1.00	24.78
ATOM	420	NH2			60	13.227	52,294	85.325		19.79
ATOM	421	N	GLY		61	7.562	51.411	89.587	1.00	21.94
ATOM	422	CA	GLY		61	7.623	52.443	90.620	1.00	22.33
ATOM	423	Ç	GLY		61	8.468	52.051	91.824		24.44
ATOM	424	ō	GLY		61	9.350	52,773	92,253		25.22
ATOM	425	N	LYS		62	8,248	50.821	92.307	1.00	26.95
ATOM	426	CA	LYS		62	9.102	50.251	93.350		26.24
ATOM	427	C	LYS		62	10.590	50.158	93.045		24.89
ATOM	428	o	LYS		62	11.443	50.668	93.756		23,23
ATOM	429	СВ	LYS		62	8.519	48.900	93.723		29.90
ATOM	430	CG	LYS		62	9.379	48.296	94.835		38.76
ATOM	431	CD	LYS		62	8.847	46.904	95.222		47.47
ATOM	432	ÇE	LYS		62	9.944	45.971	95.773		53.72
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[Drawing 18]

ATOM	433	NZ	LYS	S A	62	10.167	44.857	94.832	1.00 €	0.22
ATOM	434	N	LEU	JA	63	10.866	49.560	91.882	1.00 2	4.67
ATOM	435	CA	LEU	JA	63	12.239	49.634	91.346	1.00 2	3.65
ATOM	436	С	LEU	JA	63	12.805	51.043	91.186	1.00 2	2.74
ATOM	437	0	LEU	J A	63	13.927	51.359	91.517	1.00 2	5.19
ATOM	438	CB	LEU	J A	63	12.232	48.973	89.981	1.00 2	
ATOM	439	CG	LEU	J A	63	13.477	48.298	89.403	1.00 3	
MOTA	440	CD1	LEU	A	63	14,808	48.658	90.077	1.00 3	
ATOM	441	CD2	LEC	A	63	13.440	48.410	87.874	1.00 2	
ATOM	442	N	ALA	A	64	11.979	51.940	90.642	1.00 2	1.62
MOTA	443	CA	ALA	A	64	12.492	53.308	90.539	1.00 2	0.51
ATOM	444	C	ALA	A	64	12.862	53.971	91.863	1.00 2	
ATOM	445	0	ALA	A	64	13,890	54.636	91.984	1.00 2	
ATOM	446	CB	ALA	A	64	11.456	54.196	89.862	1.00 1	
ATOM	447	N	ALA	A	65	11.984	53.747	92.870	1.00 2	
ATOM	448	CA	ALA	. A	65	12.374	54.212	94.235	1,00 2	
ATOM	449	C	ALA	A	65	13.684	53.619	94.784	1.00 2	
ATOM	450	Q	ALA	A	65	14.551	54.328	95.268	1.00 2	
ATOM	451	CB	ALA	A	65	11.249	54.013	95.265	1.00 2	
ATOM	452	N	GLU	A	66	13.848	52.295	94.572	1.00 2	
ATOM	453	CA	GLU	A	66	15.116	51.632	94.878	1.00 2	
ATOM	454	C	GLU	A	66	16,332	52.188	94.152		6.06
ATOM	455	Q	GLU	A	66	17,321	52.604	94.744	1.00 2	
ATOM	456	CB	GLU	A	66	14.968	50.136	94.665	1.00 2	
MOTA	457	CG	GLU	A	66	13.818	49.616	95.533		1.94
MOTA	458	CD	GLU	A	66	13.546	48.142	95.293	1.00 3	
MOTA	459	OEL	GLU	A	66	13.147	47.430	96.220		0.33
MOTA	460	OE2	GLU	A	66	13.721	47.673	94.176		7.79
ATOM	461	N	ASP	A	67	16,204	52.276	92.817	1.00 24	
ATOM	462	CA	ASP	A	67	17.222	52.928	91.986		2.72
ATOM	463	C	ASP	A	67	17.549	54.333	92.402		5.72
ATOM	464	0	ASP	A	67	18.694	54.767	92.414		3.91
ATOM	465	CB	ASP	A	67	16.787	52.944	90.495	1.00 21	
ATOM	466	CG	ASP	A	67	16.824	51.580	89.801	1.00 25	
ATOM	467	OD1	ASP	A	67	17.340	50.629	90.370	1.00 23	
ATOM	468	OD2	ASP	A	67	16.349	51.434	88.666	1.00 26	
MOTA	469	N	ALA	A	68	16.485	55.059	92.773	1.00 16	
ATOM	470	CA	ALA	A	68	16.685	56.425	93.250	1.00 19	
ATOM	471	C	ALA	A	68	17.489	56.510	94.569	1.00 20	
ATOM	472	0	ALA	A	68	18.165	57.494	94.837	1.00 22	.07
ATOM	473	CB	ALA	A	68	15.330	57.134	93.419		.81
MOTA	474	И	ASN	A	69	17.472	55.371	95.299		.11
ATOM	475	CA	ASN	A	69	18.330	55.262	96.514	1.00 27	
ATOM	476	С	ASN	A	69	19.816	55.042	96.273	1.00 29	
ATOM	477	0	ASN	A	69	20.646	55.304	97.140	1.00 28	
ATOM	478	CB	ASN	A	69	17,933	54.145	97.466	1.00 24	
ATOM	479	CG	ASN	A	69	16,632	54.425	98.142	1.00 25	
ATOM	480	OD1	ASN		69	16,298	55.549	98.445	1.00 26	
ATOM	481	ND2	ASN		69	15.894	53.359	98.410	1.00 30	
ATOM	482	N	LEU	A	70	20.104	54.574	95.034	1.00 25	
ATOM	483	CA	LEU	A	70	21.514	54.442	94.627	1.00 23	
ATOM	484	C	LEU	A	70	22.329	55.691	94.640	1.00 21	
MOTA	485	Ω	LEU	A	70	22.013	56.696	94.028	1.00 23	
MOTA	486	CB	LEU	A	70	21.672	53.890	93,225	1.00 22	

(Drawing 19)

MOTA	487	CG	LEU	A	70	21.078	52,512	93.095		22.16
MOTA	488	CD1	LEU		70	21.830	51.459	93.896		20.70
MOTA	489	CD2	LEU		70	21.016	52.154	91.624		23.47
ATOM	490	N	SER	A	71	23.450	55.563	95.304	1.00	
ATOM	491	CA	SER	A	71	24.527	56.515	95.119	1.00	22.25
ATOM	492	С	SER	A	71	25.355	56.171	93.888	1.00	20.52
MOTA	493	O	SER	A	71	25.269	55.081	93.357	1.00	23.70
ATOM	494	CB	SER	A	71	25.453	56.521	96.349		22.74
ATOM	495	OG	SER	A	71	26.232	55.303	96.432		28.68
MÒTA	496	N	SER	A	72	26.220	57.079	93.445	1.00	20.02
ATOM	497	CA	SER	A	72	27.096	56.747	92.294	1.00	20.88
ATOM	498	C	SER	А	72	27.860	55.479	92.410		22,52
ATOM	499	0	SER	A	72	27.979	54.663	91.518	1.00	21.65
ATOM	500	CB	SER	A	72	28.113	57.834	92.083		19.05
ATOM	501	OG	SER	A	72	27.352	58.966	91.735	1.00	22.00
ATOM	502	N	GLY	A	73	28.336	55.318	93.640	1.00	20.71
ATOM	503	CA	GLY	A	73	28.979	54.068	94.006	1.00	16.81
ATOM	504	C	GLY	A	73	28.146	52.783	93.939	1.00	15.97
ATOM	505	O	GLY	A	73	28.697	51.705	93,753	1.00	20.02
ATOM	506	N	GLY	A	74	26.818	52.915	94.046	1.00	16.07
ATOM	507	CA	GLY	A	74	26.090	51.649	93.967	1.00	18.17
ATOM	508	C	GLY		74	25.671	51.260	92.526	1.00	21.98
ATOM	509	0	GLY	A	74	25.202	50.164	92,238	1.00	21.28
MOTA	510	N	VAL	A	75	25.887	52.210	91.567	1.00	22.48
MOTA	511	CA	VAL	A	75	25.521	51.777	90.174	1.00	22.71
ATOM	512	С	VAL	A	75	26.174	50.493	89.628	1.00	18.50
MOTA	513	0	VAL	A	75	25.497	49.573	89.210	1.00	20.32
ATOM	514	CB	VAL		75	25.820	52.946	89,218	1.00	23.52
ATOM	515	CG1	VAL	Α	75	25.719	52.707	87.712	1.00	21.49
ATOM	516	CG2	VAL	A	75	25.153	54.265	89,560	1.00	17.54
ATOM	517	N	ALA		76	27.517	50.394	89.738	1.00	21.05
ATOM	518	CA	ALA	A	76	28.149	49.125	89.372	1.00	20.51
MOTA	519	C	ALA		76	27.414	47.875	89.826	1.00	24.64
ATOM	520	0	ALA		76	27.033	47.028	89.015	1.00	24.90
ATOM	521	СВ	ALA		76	29.612	49.071	89.810	1.00	19.14
ATOM	522	N	ASN		77	27.131	47.820	91.145	1.00	20.97
ATOM	523	CA	ASN		77	26.463	46.601	91.622	1.00	17.62
ATOM	524	С	ASN		77	25.019	46.464	91,205	1.00	16.57
ATOM	525	0	ASN		77	24.536	45.350	91.024	1.00	19.24
ATOM	526	CB	ASN		77	26.615	46.509	93,137	1.00	23.52
ATOM	527	CG	ASN		77	25.817	45.362	93.677	1.00	21.57
ATOM	528	OD1	ASN		77	24.672	45.508	94.079	1.00	26.66
ATOM	529	ND2	ASN		77	26.435	44.202	93,627	1.00	24.64
ATOM	530	N	ALA		78	24.377	47.638	91.017	1.00	17.23
ATOM	531	CA	ALA		78	23.060	47.710	90.339	1.00	18.62
ATOM	532	C	ALA		78	22.874	47.025	88.941		19.92
ATOM	533	0	ALA		78	21.767	46.705	88.517		21.04
ATOM	534	CB	ALA		78	22,636	49.160	90.208		13.62
ATOM	535	N	PHE		79	24.025	46.748	88.292		19.22
ATOM	536	CA	PHE		79	24.019	45.921	87.070		20.71
ATOM	537	C	PHE		79	24.117	44.420	87.238		23.30
MOTA	538	o	PHE		79	24.161	43.662	86.273		23.64
MOTA	539	СВ	PHE		79	25.116	46.352	86.082		18.00
ATOM	540	CG	PHE		79	24.821	47.683	85.382		19.59
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[Drawing 20]

ATOM	541		PHE		79	25.181	48.903	85.984	1.00 17.05
MOTA	542		PHE		79	24.214	47.687	84.104	1.00 18.56
ATOM	543		PHE		79	25.026	50.119	85.298	1.00 19.06
ATOM	544		PHE	A	79	24.040	48.906	83.419	1.00 15.97
ATOM	545	cz	PHE		79	24.500	50.104	83.989	1.00 17.87
ATOM	546	N	SER	. A	80	24.150	43.947	88.488	1.00 19.74
MOTA	547	CA	SER	A	80	24.500	42.511	88.632	1.00 17.29
MOTA	548	C	SER	A	80	23.601	41.501	87.944	1.00 18.57
ATOM	549	0	SER	A	80	23.977	40.494	87.339	1.00 22.38
ATOM	550	CB	SER	A	80	24.608	42.106	90.125	1.00 15.17
MOTA	551	OG	SER	A	80	25.646	42.920	90.700	1.00 17.32
ATOM	552	N	GLY	A	81	22.309	41.852	88.041	1.00 19.68
MOTA	553	ÇA	GLY	A	81	21.271	41.005	87.413	1.00 22.50
ATOM	554	Ç	GLY	A	81	21.293	40.977	85.855	1.00 24.85
MOTA	555	0	GLY	A	81	21.318	39.939	85.211	1.00 23.46
ATOM	556	N	ALA	A	82	21.380	42.197	85.279	1.00 24.60
ATOM	557	CA	ALA	A	82	21.686	42.339	83.855	1.00 24.50
ATOM	558	C	ALA	A	82	22.985	41.643	83.417	1.00 25.22
MOTA	559	0	ALA	A	82	23.000	40.873	82.468	1.00 23.42
ATOM	560	CB	ALA	A	82	21.649	43.819	83.470	1.00 20.35
ATOM	561	N	PHE	A	83	24.050	41,874	84.197	1.00 23.76
ATOM	562	CA	PHE	A	83	25.319	41.242	83.894	1.00 21.76
ATOM	563	C	PHE	A	83	25.325	39.726	83.974	1.00 22.85
ATOM	564	Q	PHE	A	83	26.090	39.052	83.322	1.00 25.72
ATOM	565	CB	PHE	A	83	26.349	41.867	84.792	1.00 20.01
ATOM	566	CG	PHE	Α	83	27.770	41.527	84.394	1.00 20.95
ATOM	567	CD1	PHE	A	83	28.486	40.587	85.152	1.00 16.94
MOTA	568	CD2	PHE	A	83	28.391	42,208	83.307	1.00 23.09
ATOM	569	CE1	PHE	A	83	29.841	40.355	84.843	1.00 18.79
ATOM	570	CE2	PHE	A	83	29,751	41.991	83.001	1.00 20.95
MOTA	571	CZ	PHE	A	83	30.474	41.069	83.795	1.00 21.43
ATOM	572	N	GLY	A	84	24.409	39.187	84.768	1.00 22.31
MOTA	573	CA	GLY	A	84	24.478	37.740	84.865	1.00 24.39
MOTA	574	С	GLY	A	84	25,199	37.163	86.093	1.00 29.49
ATOM	575	O	GLY	A	84	25.158	35.963	86.362	1.00 32.27
ATOM	576	N	SER	A	85	25.873	38.058	86.843	1.00 30.33
ATOM	577	CA	SER	A	85	26.685	37.625	88.001	1.00 28.46
ATOM	578	Ç	SER	A	85	27.047	38.788	88.936	1.00 29.06
ATOM	579	0	SER	А	85	26.915	39.945	88.556	1.00 29.00
ATOM	580	CB	SER	A	85	27.915	36.861	87.536	1.00 24.04
ATOM	581	OG	SER	A	85	28.903	37.746	87.028	1.00 28.11
ATOM	582	N	PRO	A	86	27.436	38.518	90.216	1.00 29.45
ATOM	583	CA	PRO	A	86	27.599	39.650	91.122	1.00 26.27
ATOM	584	C	PRO	A	86	28.721	40.513	90.733	1.00 22.75
ATOM	585	0	PRO	A	86	29.830	40.064	90.530	1.00 22.43
ATOM	586	СВ	PRO		86	27.873	39.029	92.493	1.00 27.52
ATOM	587	CG	PRO	A	86	27.284	37.627	92.399	1.00 27.61
ATOM	588	CD	PRO	A	86	27.591	37.258	90.945	1.00 31.08
ATOM	589	N	ILE		87	28.350	41.776	90.659	1.00 22.99
ATOM	590	CA	ILE		87	29.363	42.816	90.469	1.00 24.80
ATOM	591	C	ILE		87	29.642	43.494	91.811	1.00 25.76
ATOM	592	0	ILE		87	28.956	44.424	92.220	1.00 24.68
ATOM	593	CB	ILE		87	28.908	43.860	89.427	1.00 24.26
ATOM	594	CG1	ILE		87	28.626	43.165	88.076	1.00 23.29

[Drawing 21]

ATOM	595	CG2			87	29.997	44.923	89.306		23.62
ATOM	596	CD1	ILE	A	87	27.925	44.100	87.092	1.00	
MOTA	597	N	THR	A	88	30.655	42.914	92.481	1.00	25.86
ATOM	598	CA	THR		88	30.925	43.247	93.903		25.48
MOTA	599	C	THR	A	88	32.418	43.249	94.166	1.00	25.25
MOTA	600	0	THR	A	88	33.131	42.561	93.445	1.00	24.26
MOTA	601	CB	THR	A	88	30.332	42.211	94.859	1.00	22.31
ATOM	602	OG1	THR	A	88	31.102	41.029	94.702	1.00	25.42
ATOM	603	CG2	THR	A	88	28.833	41.943	94.710	1.00	19.45
MOTA	604	M	GLU	A	89	32.891	43.970	95.204	1.00	26.63
ATOM	605	CA	GLU	A	89	34.322	43.845	95.577	1.00	25.29
ATOM	606	C	GLU	A	89	34.810	42.429	95.889	1.00	25,30
ATOM	607	0	GLU	A	89	35.924	41.999	95.611	1.00	26.30
ATOM	608	CB	GLU	A	89	34.652	44.773	96.741	1.00	25.64
MOTA	609	CG	GLU	A	89	34.334	46.193	96.340	1.00	26.52
ATOM	610	CD	GLU	A	.89	34.551	47.228	97.414	1.00	29.70
ATOM	611	OE1	GLU	A	89	35.136	48.245	97.123	1.00	33.05
ATOM	612	OE2	GLU	A	89	34.138	47.077	98.540	1.00	27.59
ATOM	613	N	LYS	A	90	33.860	41.697	96.459	1.00	26.25
ATOM	614	ÇA	LYS	A	90	34.095	40.310	96.883	1,00	28.81
ATOM	615	С	LYS	A	90	34.285	39.313	95.780	1.00	28.19
ATOM	616	0	LYS	A	90	35.206	38.518	95.773	1.00	30.49
ATOM	617	ÇВ	LYS	A	90	32.889	39.869	97.672	1.00	31.00
ATOM	618	CG	LYS	A	90	32.956	38.478	98.228	1.00	37.00
ATOM	619	CD	LYS	A	90	31.536	38.026	98.583	1.00	43.53
ATOM	620	ÇE	LYS	A	90	31.386	36.504	98.712	1.00	50.17
ATOM	621	NZ	LYS	A	90	32.257	35.875	97.701	1.00	60.80
ATOM	622	N	ASP	A	91	33.324	39.416	94.870	1.00	28.68
ATOM	623	CA	ASP	A	91	33.271	38.504	93.738	1.00	29.34
ATOM	624	Ç	ASP	A	91	33.911	38.947	92.420	1.00	29.37
ATOM	625	0	ASP	A	91	34.429	38.173	91.635	1.00	31,54
ATOM	626	CB	ASP	A	91	31.827	38.162	93.438	1.00	30.91
ATOM	627	CG	ASP	A	91	31.087	37.722	94.674	1.00	31.69
ATOM	628	QD1	ASP	A	91	31.395	36.657	95,212	1.00	35.66
ATOM	629	QD2	ASP	A	91	30.186	38.438	95.088	1.00	29.63
ATOM	630	N	ALA	A	92	33.830	40.240	92.165	1.00	28.62
ATOM	631	CA	ALA	A	92	34.443	40.713	90,919	1.00	26.75
ATOM	632	C	ALA	A	92	35.255	41,981	91.131	1.00	26.94
ATOM	633	0	ALA	A	92	34.937	43.081	90.690	1.00	26.94
ATOM	634	CB	ALA	A	92	33,390	40.934	89.817	1.00	23.93
ATOM	635	N	PRO	A	93	36.336	41.826	91.930	1.00	27,16
ATOM	636	CA	PRO	A	93	37.151	43.015	92.274	1.00	25.78
ATOM	637	C	PRO	Α	93	37.832	43.865	91.160	1.00	24.62
ATOM	638	0	PRO	A	93	37.844	45.098	91.178	1.00	22.75
ATOM	639	CB	PRO	Α	93	38.120	42.411	93.292	1.00	25.31
ATOM	640	CG	PRO	A	93	38.219	40.926	92.945		23.54
ATOM	641	CD	PRO	A	93	36.817	40.578	92.534	1.00	25.29
ATOM	642	N	ALA		94	38,409	43.174	90.170		25.16
ATOM	643	CA	ALA		94	38.954	43.886	89,005		23.77
ATOM	644	C	ALA		94	37.923	44.715	88.249		17,72
ATOM	645	0	ALA		94	38.116	45.897	88.005		19.91
ATOM	646	CB	ALA		94	39.676	42.931	88.058		21.52
ATOM	647	N	LEU		95	36.787	44.081	88.026	1.00	19.68
ATOM	648	CA	LEU		95	35.577	44.770	87.539	1.00	

[Drawing 22]

ATOM	649	С	LEU			35.001	45.902	88.385		21.85
ATOM	650	0	LEU	A	. 95	34.809	47.026	87.943	1.00	21.66
MOTA	651	CB	LEU	Α	95	34.466	43.755	87.271		20.70
ATOM	652	CG	LEU	A		33.250	44.285	86.467		21.32
MOTA	653	CD1	LEU	A	95	32.299	43.149	86.063	1.00	20.10
ATOM	654	CD2	LEU	A	95	33.698	45.111	85.240	1.00	20.93
ATOM	655	N	HIS	A	96	34.755	45.606	89.667	1.00	21.70
ATOM	656	CA	HIS	A	96	34.313	46.691	90.543	1.00	17.84
ATOM	657	C	HIS	A	96	35.214	47,924	90.580	1.00	17.76
ATOM	658	0	HIS	A	96	34.767	49.069	90.496		19.44
ATOM	659	CB	HIS	A	96	34.042	46.116	91.937	1.00	20.62
ATOM	660	CG	HIS	A	96	32.934	46.893	92.613	1.00	21.96
ATOM	661	ND1	HIS	A	96	33.021	48.094	93.229	1.00	21.34
ATOM	662	CD2	HIS	A	96.	31.614	46.470	92.680	1.00	24.62
ATOM	663	CEL	HIS	A	96	31.790	48.442	93.690		18.16
ATOM	664	NE2	HIS	A	96	30.923	47.437	93.338	1.00	23.59
ATOM	665	N	LYS	A	97	36.539	47.639	90.629	1.00	18,11
ATOM	666	CA	LYS	Α	97	37.544	48.713	90.581		18,50
ATOM	667	C	LYS	A	97	37.519	49.564	89.317		20.80
ATOM	668	0	LYS	A	97	37.452	50.781	89.373		21.08
ATOM	669	CB	LYS		97	38.924	48.085	90,766		17.26
ATOM	670	CG	LYS		97	40.125	49.014	90.594		21.24
ATOM	671	CD	LYS		97	40.283	50.213	91.525		30.10
ATOM	672	CE	LYS		97	41.482	51.160	91.164		33.55
ATOM	673	NZ	LYS		97	41.557	52.449	91.915		29.55
ATOM	674	N	LEU		98	37.532	48.857	88.155		22.36
ATOM	675	CA	LEU		98	37,291	49.555	86.851		22.04
ATOM	676	С	LEU		98	36.128	50.581	86.806		17.59
ATOM	677	0	LEU		98	36.223	51.763	86.522		18.13
ATOM	678	CB	LEU		98	37.025	48.477	85.780		21.44
ATOM	679	CG	LEU		98	36.766	49.042	84.375		20.93
ATOM	680	CD1	LEU		98	36.265	47.902	83.493		22.92
ATOM	681	CD2	LEU		98	37.963	49.801	83.811		18.27
ATOM	682	N	LEU		99	34,977	50.024	87.188		19.16
ATOM	683	CA	LEU		99	33.753	50.802	87.186		18.37
ATOM	684	C	LEU		99	33.644	51.930	88.169		20.61
ATOM	685	0	LEU		99	33.068	52.964	87.883		18.31
ATOM	686	CB	LEU		99	32.545	49.874	87.263		18.90
ATOM	687	CG	LEU		99	32.428	48.860	86.191		20.87
ATOM	688	CD1	LEU		99	32.464	49.503	84.841		14.73
ATOM	689	CD2	LEU		99	31,190	48.099	86.497		19.82
ATOM	690	N	THR		100	34.252	51.692	89.359	1.00	
ATOM	691	CA	THR		100	34.357	52.777	90.360	1.00	
ATOM	692	C	THR			35.259	53.938	89.957	1.00	
ATOM	693	Ö	THR			34.984	55.118	90.136	1.00	
ATOM	694	СВ	THR			34.889	52,174	91.674	1.00	
ATOM	695	OG1	THR			34.030	51,113	92.091	1.00	
ATOM	696	CG2	THR			35.092	53.221	92.758	1.00	
ATOM	697	N	ASN			36.397	53.537	89.358	1.00	
ATOM	698	CA	ASN			37.415	54.515	88.998		19.91
ATOM	699	C	ASN			37.022	55.479	87.873	1.00	
ATOM	700	0	ASN			37.610	56.546	87.711	1.00	
ATOM	701	CB	ASN			38,692	53.763	88.716	1.00	
ATOM	702	CG	ASN			39.950				
	142	O.G	42 DIA		TAT	23.320	54.556	89.041	1.00	c4.30

[Drawing 23]

MOTA	703	OD1	ASN	A	101	40.938	53.978	89.459	1.00 30.43
ATOM	704	ND2	ASN			39.964	55.868	88.889	1.00 23.45
MOTA	705	И	MET			35.952	55.090	87.154	1.00 21.94
MOTA	706	CA	MET			35.407	55.984	86.103	1.00 22.28
ATOM	707	С	MET			34.142	56.755	86.455	1.00 22.36
ATOM	708	Q	MET			33.571	57.482	85.638	1.00 23.37
ATOM	709	CB	MET	A	102	35.162	55.213	84.781	1.00 19.03
ATOM	710	CG	MET			34.239	54.001	84.972	1.00 18.05
ATOM	711	SD	MET	A	102	33.744	53.082	83.481	1.00 20.07
ATOM	712	CE	MET			32.429	54.165	83.010	1.00 16.04
ATOM	713	N	ILE			33.681	56.555	87.724	1.00 21.00
MOTA	714	CA	ILE	A	103	32.441	57,221	88.180	1.00 18.78
ATOM	715	С	ILE	A	103	32.371	58.697	87.833	1.00 17.38
ATOM	716	0	ILE	A	103	31.413	59.152	87.245	1.00 18.08
ATOM	717	CB	ILE	A	103	32.174	57.025	89.732	1.00 16.18
ATOM	718	CG1	ILE .	A	103	31.696	55.603	90.031	1.00 19.21
ATOM	719	CG2	ILE	A	103	31.135	58.037	90.272	1.00 12.63
ATOM	720	CD1	ILE	A	103	31.708	55.185	91.522	1.00 17.58
ATOM	721	N	GLU .	A	104	33.426	59.429	88.218	1.00 18.81
ATOM	722	CA	GLU .	A	104	33.369	60.900	88.092	1.00 18.36
ATOM	723	C	GLU	A	104	33.828	61.520	86.772	1.00 19.60
MOTA	724	0	GLU	A	104	33.420	62.606	86.365	1.00 19.13
ATOM	725	CB	GLU .	A	104	34.092	61.600	89.241	1.00 18.56
ATOM	726	CG	GLU	A	104	33.446	61.448	90.617	1.00 19.21
MOTA	727	CD	GLU .	A	104	31.994	61.944	90.665	1.00 23.36
ATOM	728	OE1	GLU	A	104	31.225	61.359	91.382	1.00 26.94
ATOM	729	OE2	GLU .	A	104	31.574	62.888	90.013	1.00 28.46
ATOM	730	N	ASP .	A	105	34.606	60.713	86.049	1.00 18.95
ATOM	731	CA	ASP .	A	105	34.743	60.936	84.587	1.00 17.40
ATOM	732	C	ASP .	A	105	33.378	61.099	83.886	1.00 15.12
MOTA	733	0	ASP	A	105	33.104	62.102	83.234	1.00 18.61
MOTA	734	CB	ASP .	A	105	35.429	59.743	83.951	1.00 16.81
ATOM	735	CG	ASP .	A	105	36.831	59.545	84.440	1.00 15.10
ATOM	736	OD1	ASP .	A	105	37.573	60.520	84.573	1.00 19.01
ATOM	737	OD2	ASP .	A	105	37.177	58.402	84.685	1.00 15.56
ATOM	738	N	ALA .	A	106	32.500	60.091	84.096	1.00 15.05
MOTA	739	ÇA	ALA.	Α	106	31.111	60.157	83.607	1.00 15.61
MOTA	740	С	ALA .	A	106	30.166	61.126	84.315	1.00 19.62
ATOM	741	0	ALA.	A	106	29.409	61.881	83.720	1.00 18.83
ATOM	742	CB	ALA	A	106	30.467	58.782	83.682	1.00 11.73
ATOM	743	N	GLY	A	107	30.263	61.102	85.674	1.00 21.49
MOTA	744	CA	GLY .	A	107	29.323	61.899	86.503	1.00 16.83
ATOM	745	C	GLY .	A	107	29.599	63.356	86.594	1.00 14.80
ATOM	746	0	GLY	A	107	28.714	64.204	86.575	1.00 17.67
MOTA	747	N	ASP	A	108	30.899	63.611	86.662	1.00 16.37
ATOM	748	CA	ASP .	A	108	31.305	65.002	86.772	1.00 17.18
ATOM	749	С	ASP .	A	108	31.877	65.572	85.485	1.00 18.56
ATOM	750	0	ASP	A	108	31.324	66.472	84.877	1.00 18.77
ATOM	751	CB	ASP .	A	108	32.282	65.144	87.947	1.00 16.92
MOTA	752	CG	ASP .	A	108	32.862	66.530	88.143	1.00 21,34
ATOM	753	OD1	ASP	A	108	32,247	67.528	87.812	1.00 23.69
ATOM	754	QD2	ASP	A	108	33,983	66.642	88.614	1.00 27.32
MOTA	755	N	LEU			33,049	65.065	85.107	1.00 18.48
MOTA	756	CA	LEU	A	109	33.814	65.703	84.005	1.00 20.77

[Drawing 24]

ATOM	757	C	LEU	A	109	32.954	65.922	82.752	1.00	21.46
ATOM	758	0	LEU	A	109	32.905	67.026	82.199	1.00	23.24
ATOM	759	CB	LEU	A	109	35.024	64.826	83.679		20.60
ATOM	760	CG			109	36.393	65.285	84.113		21.65
ATOM	761	CD1	LEU	A	109	37.408	64.213	84.096	1.00	17.20
ATOM	762	CD2	LEU	A	109	36.469	66.186	85.254		20.25
ATOM	763	N			110	32.206	64.812	82.422	1.00	21.81
ATOM	764	CA			110	31.336	64.771	81.215	1.00	20.79
ATOM	765	C	ALA	A	110	30.074	65.608	81.232		23.23
MOTA	766	0			110	29.504	65.885	80.182		23.66
ATOM	767	CB			110	30.921	63.333	80.868	1.00	20.49
MOTA	768	N	THR	A	111	29.672	66,038	82.465		20.15
MOTA	769	ÇA	THR	A	111	28.453	66.863	82.653	1.00	19.21
MOTA	770	C	THR	A	111	28.654	68.292	83.132	1.00	18.58
MOTA	771	0	THR	A	1.1.1	27.754	69.106	83.131	1.00	18.77
MOTA	772	CB	THR	A	111	27.469	66.257	83.628	1.00	18.46
ATOM	773	OG1	THR	A	111	28.011	66.399	84.949	1.00	23.25
ATOM	774	CG2	THR	A	111	27.094	64.802	83.347	1.00	15.78
ATOM	775	N	ARG	A	112	29.870	68.595	83.547	1.00	20.94
MOTA	776	CA			112	30.068	69.805	84.369	1.00	22.62
ATOM	777	С	ARG	A	112	29.745	71.185	83.786	1.00	23.68
ATOM	77B	0	ARG			29.035	72.025	84.325		21.09
ATOM	779	CB	ARG	A	112	31.512	69.782	84.911	1.00	22.88
ATOM	780	CG	ARG			31.847	70.852	85.952	1.00	22.67
ATOM	781	CD	ARG			33.319	70.922	86.319		18.55
ATOM	782	NE	ARG	A	112	33.831	69.709	86.930	1.00	22.11
ATOM	783	CZ	ARG			35.138	69.496	86,853	1.00	21.99
MOTA	784	NHT	ARG	A	112	35.949	70.322	86.227		23.29
ATOM	785	NH2	ARG			35.623	68.436	87.414	1.00	23.83
MOTA	786	N	SER	A	113	30.323	71.398	82.583	1.00	21.66
ATOM	787	CA	SER			30.146	72.736	81.981	1.00	19.14
ATOM	788	C	SER			28.721	73.124	81.629	1.00	20.09
ATOM	789	О	SER			28.288	74.263	81.806		23.06
ATOM	790	CB	SER			31.029	72.919	80.732		24.64
ATOM	791	OG	SER			30.812	71.854	79.778		25.18
ATOM	792	N	ALA			27.955	72.094	81.186	1.00	19.43
ATOM	793	CA	ALA			26.510	72.272	80.944	1.00	17.15
MOTA	794	C	ALA			25.695	72.377	82.247		16.95
ATOM	795	0	ALA			24.890	73.293	82,402		17.79
ATOM	796	CB	ALA			25.935	71.096	80.117		15.05
ATOM	797	N	LYS			25.993	71.462	83.200		18.76
ATOM	798	CA	LYS			25.431	71.618	84.559		20.52
ATOM	799	C	LYS			25.524	73.029	85.143		18.24
ATOM	800	0	LYS			24.535	73.710	85.429		19.61
ATOM	801	CB	LYS			26.048	70.606	85.508	1.00	
ATOM	802	CG	LYS			25.304	69.294	85,482		22.93
ATOM	803	CD	LYS			25.867	68.477	86.654		26.73
ATOM	804	CE	LYS			25.353	67.039	86.850	1.00	
MOTA	805	NZ	LYS			23.888	67.023	87.009	1.00	
ATOM	806	N	ASP			26.784	73.454	85,203	1.00	
ATOM	807	CA.	ASP			27.073	74.739	85.832	1.00	
ATOM	808	С	ASP			26.589	75.938	85.076	1.00	
ATOM	809	O O	ASP			26.208	76.967	85.612	1.00	
ATOM	810	CB	ASP	A	TTP	28.573	74.896	86.053	1.00	22.26

(Drawing 25)

ATOM	811	CG	ASP			29.203	73.871	87.029	1.00 25.80
MOTA	812		ASP			28.502	73.085	87.657	1.00 27.77
MOTA	813		ASP			30.431	73.847	87.166	1.00 28.44
ATOM	814	N	HIS			26.596	75.793	83.741	1.00 23.53
ATOM	815	CA	HIS			26.022	76,894	82.957	1.00 21.71
ATOM	816	С	HIS			24.496	77.044	83.026	1.00 20.75
ATOM	817	0	HIS	A	117	23.932	78.108	83.223	1.00 20.51
ATOM	818	CB	HIS	A	117	26.536	76.787	81.501	1.00 22.93
ATOM	819	CG	HIS	A	117	25.987	77.909	80.635	1.00 22.08
ATOM	820	NDI	HIS	A	117	26.531	79.128	80.459	1.00 23.33
ATOM	821	CD2	HIS	A	117	24.824	77.852	79.881	1.00 22.30
ATOM	822	CE1	HIS	А	117	25.741	79.836	79.615	1.00 23.49
ATOM	823	NE2	HIS	А	117	24.693	79.041	79.262	1.00 23.57
ATOM	824	N	TYR	A	118	23.825	75.906	82.825	1.00 19.95
ATOM	825	CA	TYR	A	118	22.363	76.013	82.795	1.00 19.91
ATOM	826	C	TYR	A	118	21,711	76.075	84.159	1.00 22.89
ATOM	827	0	TYR	A	118	20,615	76.596	84.278	1.00 22.53
MOTA	828	CB	TYR	A	118	21.702	74.869	82.020	1.00 18.70
ATOM	829	CG	TYR			22.112	74.964	80.550	1.00 19.86
MOTA	830	CD1	TYR			21.604	76.030	79.775	1.00 20.81
MOTA	831	CD2	TYR			22.998	74.004	80.005	1.00 20.19
ATOM	832	CEL	TYR			21.964	76.104	78.410	1.00 24.18
MOTA	833	CE2	TYR			23.393	74.097	78.652	1,00 22.07
ATOM	834	CZ	TYR			22.841	75.133	77.869	1.00 24.85
ATOM	835	OH	TYR			23.138	75.231	76.525	1.00 25.02
ATOM	836	N	MET			22.385	75,453	85.158	1.00 22.72
ATOM	837	CA	MET			21.795	75.408	86.516	1.00 25.49
ATOM	838	C.	MET			20.328	75.033	86.619	1.00 22.79
ATOM	839	o o	MET			19.526	75.639	87.309	1.00 23.65
ATOM	840	CB	MET			22.009	76.758	87.200	1.00 31.90
ATOM	841	CG	MET			23.479	77.200	87,296	1.00 41.79
ATOM	842	SD	MET			23.683	78.779	88.163	1.00 50.25
ATOM	843	CE	MET			22.932	79.838	86.910	1.00 48.37
ATOM	844	N	ARG			19.958	74.021	85.840	1.00 21.05
ATOM	845	CA	ARG			18.529	73.782	85.704	1.00 19.45
	846	C	ARG			17.877	73.247	86.989	1.00 16.34
MOTA	847	ō	ARG			18.483	72.369	87.587	1.00 17.21
MOTA		CB	ARG			18.345	72.757	84.558	1.00 16.25
ATOM	848					16.913	72.517	84.063	1.00 17.39
MOTA	849	CG	ARG			16.775	71.558	82.842	1.00 17.33
MOTA	850	CD					71.636	82.189	1.00 19.09
ATOM	851	NE	ARG			15.450		81.479	1.00 20.39
ATOM	852	CZ	ARG			14.929	70.642		1.00 12.14
ATOM	853	NH1				15.600	69.574	81.259	1.00 12.74
ATOM	854		ARG			13.724	70.767	81.007	
ATOM		N	ILE			16.676	73.723	87.290	
ATOM	856	CA	ILE			15.807	73.186	88.360	1.00 22.91
ATOM	857	C	ILE			15.258	71.795	88.080	1.00 23.50
MOTA	858	0	ILE			14.648	71.571	87.043	1.00 26.14
ATOM	859	ÇB	ILE			14.594	74.134	88.686	1.00 25.98
ATOM	860	CG1				14.986	75.592	88.783	1.00 29.16
ATOM	861	CG2	ILE			13.793	73.870	89.982	1.00 23.20
ATOM	862	CD1	ILE			16.075	75.914	89.785	1.00 29.73
ATOM	863	И	ARG			15.463	70.877	89.036	1.00 21.01
ATOM	864	CA	ARG	A	122	14.883	69.534	88.976	1.00 19.80

[Drawing 26]

ATOM	865	C	ARG			13.372	69.532	89.091		17.61
MOTA	866	0	ARG	A	122	12.805	70.488	89.613	1.00	
ATOM	867	CB	ARG	A	122	15.533	68.653	90.033	1.00	
MOTA	868	CG	ARG			17.023	68.706	89.841	1.00	
ATOM	869	CD	ARG	A	122	17.721	67.861	90.852	1.00	21.00
ATOM	870	NE	ARG			19.141	68.121	90.748	1.00	28.80
ATOM	871	CZ	ARG	A	122	19.914	67.081	90.491	1.00	34.31
ATOM	872	NH1	ARG	A	122	19.401	65.871	90.426	1.00	40.28
MOTA	873	NH2	ARG	A	122	21.196	67,257	90.289	1.00	32.47
ATOM	874	N	PRO	A	123	12.700	68.460	88.577	1.00	17.64
ATOM	875	CA	PRO	A	123	11.243	68.461	88.684	1.00	18.56
ATOM	876	C	PRO	A	123	10.668	68.630	90.118	1.00	20.86
ATOM	877	0	PRO	A	123	9.881	69.536	90.331	1.00	20.65
ATOM	878	ÇВ	PRO	A	123	10.808	67.150	88.004	1.00	16.86
ATOM	879	CG	PRO	A	123	12.028	66.572	87.296	1.00	15.55
ATOM	880	CD	PRO	A	123	13.235	67.291	87.881	1.00	15.48
ATOM	881	N	PHE	A	124	11.077	67.771	91.105	1.00	21.82
ATOM	882	CA	PHE	A	124	10.489	67.948	92.468	1.00	20.01
ATOM	883	C	PHE	A	124	10.541	69.394	93.016	1.00	16.73
ATOM	884	0	PHE	A	124	9.581	69.970	93.486	1.00	17.82
ATOM	885	CB	PHE .	A	124	11.044	66.869	93.422	1.00	17.84
ATOM	886	CG	PHE .	A	124	12.484	67.199	93.795	1.00	19.87
ATOM	887	CD1	PHE .	A	124	12.748	68.117	94.850	1.00	20.96
ATOM	888	CD2	PHE .	A	124	13,554	66.632	93.075	1.00	19.87
ATOM	889	CEI	PHE .	A	124	14.068	68.524	95.134	1.00	21.78
ATOM	890	CEZ	PHE .	A	124	14.881	67.014	93.381	1.00	21,98
ATOM	891	CZ	PHE .	Α	124	15.129	67.975	94.386	1,00	23.27
ATOM	892	N	ALA .	A	125	11,681	70.039	92.775	1.00	18.30
ATOM	893	CA	ALA :	Ά	125	11.866	71.464	93.089	1.00	20.06
ATOM	894	C	ALA.	A	125	11.033	72.481	92.291	1.00	24.90
ATOM	895	0	ALA.	A.	125	10.455	73.445	92.789	1.00	24.77
ATOM	896	CB	ALA	A	125	13.358	71.840	92.990	1.00	16.96
ATOM	897	N	PHE :	A	126	10.941	72.202	90.977	1.00	23.91
ATOM	898	CA	PHE .	A	126	10.017	72.958	90.145	1.00	22.66
ATOM	899	С	PHE :	A	126	8.590	72.919	90.692	1.00	20.74
ATOM	900	O	PHE :	A	126	7.910	73.945	90.785	1.00	21.66
ATOM	901	СВ	PHE :	A	126	10.051	72.379	88.705	1.00	19.61
ATOM	902	CG	PHE 2	A	126	9.147	73.140	87.765	1.00	16.99
ATOM	903	CD1	PHE 2	A	126	9,669	74.211	87.022	1.00	15.15
ATOM	904	CD2	PHE 2	A	126	7.794	72.757	87.656	1.00	17.75
ATOM	905	CE1	PHE 2	A	126	8.824	74.913	86.144	1.00	14.16
ATOM	906	CE2	PHE 2	Ą	126	6.940	73.472	86.799	1.00	17.56
ATOM	907	CZ	PHE 2	A	126	7.471	74,538	86.048	1.00	12.93
ATOM	908	N	TYR 2	A	127	8.183	71.664	91.002	1.00	20.13
ATOM	909	CA	TYR 2	A	127	6.843	71.414	91.525	1.00	20.41
ATOM	910	C	TYR I	Ą	127	6.642	71.689	93.032	1.00	24.41
ATOM	911	0	TYR A	A	127	5.525	71.665	93.532	1.00	23.70
ATOM	912	CB	TYR A	A	127	6.370	69.994	91.207		20.85
ATOM	913	CG	TYR A	A	127	6.198	69.850	89.697	1.00	25.51
ATOM	914	CD1	TYR A	A	127	7.200	69.185	88.947	1.00	27.02
ATOM	915	CD2	TYR A			5.064	70.407	89.065	1.00	25.24
ATOM	916	CE1	TYR A			7.057	69.037	87.552		28.75
ATOM	917	CE2	TYR A	A	127	4.907	70.261	87.663	1.00	28.24
ATOM	918	CZ	TYR A			5.891	69.543	86.936	1.00	28.76

|Drawing 27|

MOTA	919				127	5.716	69.314	85.588	1.00 27.53
ATOM	920	N			128	7.764	71.960	93.723	1.00 23.74
ATOM	921	CA			128	7.675	72.217	95.170	1.00 24.88
ATOM	922	С			128	7.138	71.011	95.924	1.00 24.57
ATOM	923	0			128	6.383	71.095	96.866	1.00 29.82
ATOM	924	N			129	7.527	69.854	95.426	1.00 24.16
ATOM	925	CA	VAL	A	129	7.144	68.604	96.044	1.00 23.21
ATOM	926	C	VAL	A	129	8.377	67.802	96.358	1.00 24.99
ATOM	927	0	VAL	A	129	9.529	68.188	96.189	1.00 25.90
ATOM	928	CB	VAL	A	129	6.193	67.776	95.187	1.00 22.38
ATOM	929	CG1	VAL	A	129	6.738	67.390	93.815	1.00 17.75
ATOM	930	ÇG2	VAL	A	129	4.895	68.549	95.125	1.00 24.52
ATOM	931	N	SER	A	130	8.089	66.617	96.832	1.00 25.98
ATOM	932	CA			130	9.242	65,724	96.973	1.00 29.71
ATOM	933	C			130	9.322	64.672	95.895	1.00 29.73
MOTA	934	Ó			130	8.403	64.485	95.103	1.00 30.28
ATOM	935	СВ			130	9.183	64.999	98.309	1.00 35.36
ATOM	936	OG			130	7.964	64.244	98.427	1.00 41,61
ATOM	937	N			131	10.440	63.952	95.934	1.00 30.35
ATOM	938	CA			131	10,533	62.819	94.996	1.00 27.90
ATOM	939	C			131	9.733	61.569	95.361	1.00 28.80
ATOM	940	0	THR			9.051	61.508	96.366	1.00 30.16
ATOM	941	CB			131	11.996	62.471	94.783	1.00 26.62
ATOM	942	OG1	THR			12.500	61.841	95.953	1.00 29.21
	943	CG2	THR			12.839	63.682	94.446	1.00 19.32
ATOM	944	N N			132	9.835	60.528	94.551	1.00 27.12
ATOM			CYS			9.203	59.271	94.996	1.00 27.12
ATOM	945	CA				9.203	58.540	96.137	1.00 27.75
ATOM	946	C	CYS CYS			9,556		96.634	1.00 28.80
ATOM	947	0					57.487		
MOTA	948	CB	CYS			9.081	58.274	93.831	1.00 24.68
MOTA	949	SG	CYS			10.538	57.273	93.459	1.00 24.12
MOTA	950	N			133	11.021	59.158	96.447	1.00 33.31
ATOM	951	CA			133	12.012	58.492	97.236	1.00 39.44
ATOM	952	C	ASN			12.008	58.776	98.750	1.00 45.19
ATOM	953	0	ASN			12.269	57.928	99.583	1.00 50.38
ATOM	954	CB			133	13.285	58.876	96.522	1.00 35.83
ATOM	955	CG	ASN			14.294	57.823	96.731	1.00 37.18
MOTA	956	OD1				15.478	58.059	96.663	1.00 38.83
MOTA	957		ASN			13.815	56.623	97.017	1.00 42.51
MOTA	958	N	THR		134	11.642	59.993	99.118	1.00 48.69
MOTA	959	CA	THR			12.585	61.110	98.925	1.00 56.17
MOTA	960	C	THR			13.935	61.171	99.735	1.00 61.49
MOTA	961	OCTI	THR	A	134	14.052		100.816	1.00 66.36
ATOM		OCT2				14.937	61.779	99.291	1.00 63.18
ATOM	963	CB	THR	A	134	11.704	62.374	98.968	1.00 56.04
ATOM	964	OG1	THR	A	134	12.306	63.614	98.469	1.00 56.23
MOTA	965	CG2	THR	A	134	10.869	62.400	100.243	1.00 53,77
MOTA	966	N	GLN	A	137	16.953	60.437	100.819	1.00100.00
ATOM	967	CA	GLN	A	137	17.845	60.498	102.027	1.00 99.78
ATOM	968	С	GLN	A	137	19.036	61.512	102.211	1,00 98.61
MOTA	969	0	GLN	A	137	19.386	61.891	103.324	1.00 97.54
MOTA	970	CB	GLN	A	137	18.343		102.397	1,00100.00
ATOM	971	CG	GLN			17.669	58.407	103.615	1.00 99.38
ATOM	972	CD	GLN			18.060		104.963	1.00 98.64

[Drawing 28]

ATOM	973		GLN			17.484		106.009	1.00 98.03
ATOM	974	NE2	GLN			19.086		104.972	1.00100.00
ATOM	975	N	ASP	A	138	19.627		101.081	1.00 97.91
ATOM	976	CA			138	20.479		101.162	1.00 95.99
ATOM	977	C	ASP	A	138	19.761		101.063	1.00 93.86
ATOM	978	0	ASP	A	138	18.589	64.579	100.711	1.00 92.76
ATOM	979	CB	ASP	A	138	21.585	63.102	100.115	1.00 97.46
ATOM	980	CG	ASP	A	138	22.893	62.963	100.866	1.00100.00
MOTA	981	OD1	ASP	A	138	23.371	61.842	101.028	1.00100.00
MOTA	982	QD2	ASP	A	138	23.432	63.969	101.333	1.00100.00
ATOM	983	N	LYS	A	139	20.494	65.564	101.380	1.00 92.69
MOTA	984	CA	LYS	A	139	19.813	66.855	101.218	1.00 91.50
ATOM	985	C	LYS	A	139	19.719	67.313	99.775	1.00 88.92
ATOM	986	0	LYS	A	139	20.557	68.082	99.308	1.00 89.47
ATOM	987	CB	LYS	A	139	20.464	67.987	102.010	1.00 94.21
ATOM	988	CG	LYS	A	139	19.574	69.246	102.020	1.00 96.82
ATOM	989	CD	LYS	A	139	20.362	70.569	102.123	1.00 99.43
ATOM	990	CE	LYS	A	139	20.785	71.241	100.793	1.00100.00
MOTA	991	NZ	LYS	A	139	21.686	70.402	99.978	1.00100.00
ATOM	992	N	LEU	A	140	18.654	66.828	99.114	1.00 85.87
ATOM	993	CA	LEU	A	140	18.426	67.018	97.660	1.00 79.60
ATOM	994	C	LEU	A	140	18.772	68.392	97.043	1.00 75.62
ATOM	995	0	LEU	A	140	18.242	69.442	97.416	1.00 75.74
ATOM	996	CB	LEU	Α	140	16.981	66.620	97.283	1.00 77.27
ATOM	997	CG	LEU	А	140	16.640	65.142	97.462	1.00 74.31
ATOM	998	CD1	LEU	A	140	17.569	64.263	96.645	1.00 73.21
ATOM	999	CD2	LEU	A	140	15.178	64.838	97.167	1.00 73.91
ATOM	1000	N	SER	A	141	19.713	68.357	96.067	1.00 70.08
ATOM	1001	CA	SER	Α	141	19.868	69.619	95.320	1.00 64.86
ATOM	1002	C	SER	A	141	18.713	69.941	94.396	1.00 61.18
ATOM	1003	0	SER	A	141	18.189	69.080	93.708	1.00 61.77
ATOM	1004	CB	SER	A	141	21.127	69.682	94.461	1.00 64.49
ATOM	1005	OG	SER	A	141	21.354	71.028	94.002	1.00 66.43
ATOM	1006	N	LYS	A	142	18.349	71.235	94.379	1.00 57.81
ATOM	1007	CA	LYS	Α	142	17.238	71.661	93.493	1.00 54.35
ATOM	1008	С	LYS	A	142	17.498	71.829	91.964	1.00 45.94
ATOM	1009	0	LYS	A	142	16.615	72.091	91,202	1.00 40.89
ATOM	1010	CB	LYS	Α	142	16.631	72.953	94.061	1.00 57.69
ATOM	1011	CG	LYS	A	142	17.518	74.222	93.959	1.00 62.63
ATOM	1012	CD	LYS	A	142	16.625	75.420	93.545	1.00 68.07
ATOM	1013	CE	LYS	A	142	17.200	76.856	93.475	1.00 71.28
MOTA	1014	NZ	LYS	A	1.42	16.136	77.844	93.162	1.00 70.88
MOTA	1015	N	ASN	Α	143	18.772	71.719	91.611	1.00 45.99
MOTA	1016	CA	ASN	A	143	19.527	72.392	90.538	1.00 44.71
ATOM	1017	C	ASN	A	143	20.592	71.481	89.878	1.00 42.57
ATOM	1018	0	ASN	A	1.43	20.794	70.322	90.283	1.00 40.17
ATOM	1019	CB	ASN	A	143	20.343	73.547	91.124	1.00 50.34
ATOM	1020	CG	ASN	Α	143	19.624	74.818	90.876	1.00 55.19
ATOM	1021	OD1	ASN	A	143	18.441	74.944	91.098	1.00 59.21
ATOM	1022	ND2				20.366	75.786	90.389	1.00 59.88
ATOM	1023	N	GLY	A	144	21.265	72.084	88.819	1.00 39.72
ATOM	1024	CA	GLY	A	144	22.264	71.401	87.958	1.00 26.74
ATOM	1025	C	GLY	A	144	21.691	70.105	87.407	1.00 21.44
ATOM	1026	0	GLY	A	144	22.343	69.071	87.322	1.00 25.78

[Drawing 29]

ATOM	1027	N	SER.	A	145	20.380	70.181	87.105	1.00	19.15
ATOM	1028	CA	SER	A	145	19.590	69.023	86.596	1.00	20.14
ATOM	1029	C	SER .			20.077	68.461	85.223		20.58
MOTA	1030	0	SER .	A	145	20.121	67.279	84.943	1.00	21.22
ATOM	1031	CB	SER .	Α	145	18.171	69.499	86.338	1.00	18.89
ATOM	1032	OG	SER .	A	145	17.219	68.526	86.712	1.00	28.02
ATOM	1033	N	TYR .	A	146	20.531	69.419	84.399	1.00	22.70
ATOM	1034	CA	TYR .	A	146	20.867	69.170	82.968	1.00	19.51
ATOM	1035	C	TYR .	Α	146	22.366	69.260	82.633	1.00	16.00
ATOM	1036	0	TYR .	A	146	22.930	70.339	82.736	1.00	17.85
ATOM	1037	CB	TYR .	A	146	20.097	70.216	82.114	1.00	18.79
ATOM	1038	CG	TYR .	A	146	20.211	69.933	80.600	1.00	20.29
ATOM	1039	CD1	TYR 3	A	146	19.310	69.027	80.015	1.00	19.58
ATOM	1040	CD2	TYR 3	A	146	21.205	70.582	79.824	1.00	20.42
ATOM	1041	CEl	TYR A	A	146	19.389	68.781	78.631	1.00	23.13
ATOM	1042	CE2	TYR I	A	146	21.287	70.337	78.424	1.00	21.68
ATOM	1043	CZ	TYR Z			20.349	69.449	77.849	1.00	20.03
ATOM	1044	ОН	TYR I	A	146	20.320	69.236	76.483	1.00	21.40
ATOM	1045	N	PRO I	A.	147	22.994	68.144	82.187	1.00	16,52
MOTA	1046	CA	PRO I	A	147	22.385	66.793	82.188	1.00	17,87
ATOM	1047	С	PRO A	4	147	22.496	66.069	83.580		20.67
ATOM	1048	0	PRO A	4	147	23.198	66.531	84.480		22.35
MOTA	1049	CB	PRO 2	4	147	23.250	66.107	81.122		15.36
ATOM	1050	CG	PRO 2			24.649	66.716	81.297		15.06
MOTA	1051	CD	PRO A			24.356	68.168	81.630	1.00	
ATOM	1052	N	SER A			21.827	64.908	83.662		18.73
MOTA	1053	CA	SER A	1	148	21.951	64.019	84.823	1.00	
ATOM	1054	С	SER I			23.269	63.281	84.930		19.93
ATOM	1055	O	SER A			23.601	62.439	84.108		20.09
ATOM	1056	CB	SER A	ì	148	20.828	62,997	84.807	1.00	18.55
ATOM	1057	OG	SER A	7	148	20.990	61.966	85,780		19.29
ATOM	1058	N	GLY A	ì	149	24.036	63.618	85.972	1.00	18.26
ATOM	1059	CA	GLY A	ł	149	25.284	62.888	86.231		16.00
ATOM	1060	C	GLY 2			25,096	61.411	86.577		19.38
ATOM	1061	0	GLY A			25.791	60.549	86.044		21.04
ATOM	1062	N	HIS A			24.085	61.096	87.427		20.25
ATOM	1063	CA	HIS A			23.690	59.680	87,624		19.01
ATOM	1064	C	HIS A			23.381	58.861	86.330		19.93
ATOM	1065	0	HIS A			23.833	57.725	86.145	1.00	20.78
ATOM	1066	CB	HIS A			22.507	59.533	88.619		18.01
ATOM	1067	CG	HIS A			22,162	58.068	88.909		20.60
ATOM	1068	ND1	HIS F			22.864	57.235	89.730		23,41
ATOM	1069	CD2	HIS A	1	150	21.117	57.313	88.374		21.91
MOTA	1070		HIS F			22,287	55.996	89,732		22.83
ATOM	1071	NE2	HIS A	4	150	21.220	56.051	88.893		24.19
ATOM	1072	N	THR A			22.593	59.482	85.432		18.98
ATOM	1073	CA	THR F			22.325	58.814	84,132		17.17
ATOM	1074	C	THR A			23.548	58.652	83.228		13.61
ATOM	1075	Ö	THR A			23.814	57.594	82.659		17.00
ATOM	1076	СВ	THR A			21.270	59.590	83.407		16.57
ATOM	1077	OG1	THR A			20.137	59.738	84.258		18.00
ATOM	1078	CG2	THR A			20.898	58.983	82.045		13.22
ATOM	1079	N	SER A			24.361	59.722	83.197		14.30
ATOM	1080	CA	SER A			25.687	59.598	82.557		15.99
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[Drawing 30]

ATOM	1081	C	SER	A	152	26.575	58.446	83.029	1.00 19.72
ATOM	1082	0	SER	A	152	27.086	57.628	82.255	1.00 17.38
MOTA	1083	CB	SER	A	152	26.434	60.927	82.644	1.00 12.58
MOTA	1084	0G	SER	A	152	27.648	60.861	81.911	1.00 16.08
ATOM	1085	N	ILE	A	153	26.662	58.340	84.403	1.00 20.60
ATOM	1086	CA	ILE	A	153	27.272	57.121	85.017	1.00 16.15
MOTA	1087	С	ILE	A	153	26.622	55.802	84.631	1.00 10.76
ATOM	1088	٥	ILE	A	153	27.293	54.850	84.262	1.00 14.38
ATOM	1089	CB	ILE	A	153	27.384	57.170	86.608	1.00 15.02
MOTA	1090	CG1	ILE	A	153	28.187	58.421	86.963	1.00 16.37
ATOM	1091	CG2	ILE	A	153	28.154	55.944	87.164	1.00 12.98
ATOM	1092	CD1			153	27,870	59.034	88.338	1.00 16.58
ATOM	1093	N	GLY	A	154	25.285	55.763	84.720	1.00 9.72
ATOM	1094	ÇA			154	24.662	54,476	84.397	1.00 13.63
ATOM	1095	C			154	24.843	54.033	82.910	1.00 16.00
ATOM	1096	0			154	25.022	52.866	82.571	1.00 16.37
MOTA	1097	N			155	24.801	55.047	82.025	1.00 16.77
MOTA	1098	CA			155	24.960	54.747	80.589	1.00 16.73
ATOM	1099	C			155	26.378	54.351	80.200	1.00 15.90
ATOM	1100	ò			155	26.656	53.301	79.628	1.00 18.41
ATOM	1101	СВ	TRP		155	24.442	55.940	79.771	1.00 16.52
ATOM	1102	CG			155	24.320	55.475	78.321	1.00 19.11
ATOM	1103	CD1			155	25.009	56.015	77.210	1.00 18.94
ATOM	1104	CD2	TRP			23.471	54.426	77.765	1.00 18.98
ATOM	1105	NE1			155	24.679	55.352	76.044	1.00 17.77
ATOM	1106	CE2			155	23.781	54.322	76.357	1.00 22.22
ATOM	1107	CE3	TRP			22.585	53.486	78.337	1.00 19.43
ATOM	1108	CZ2	TRP		155	23.084	53.377	75.564	1.00 17.99
ATOM	1109	CZ3	TRP			21.913	52.538	77.537	1.00 19.46
ATOM	1110	CH2	TRP			22.191	52.464	76.158	1.00 17.84
ATOM	1111	N	ALA			27.299	55.209	80.623	1.00 15.37
ATOM	1112	CA	ALA			28.702	54.836	80.515	1.00 14.36
ATOM	1113	С	ALA			29.156	53.503	81,108	1.00 19.46
ATOM	1114	0	ALA			29.895	52.723	80.528	1.00 19.86
ATOM	1115	CB	ALA			29.564	55.918	81.136	1.00 15.83
ATOM	1116	И	THR			28.651	53.207	82.327	1.00 19.95
ATOM	1117	CA	THR			28.820	51.832	82.831	1.00 17.52
ATOM	1118	С	THR			28,177	50.744	81.994	1.00 15.22
ATOM	1119	0	THR			28.825	49.745	81.765	1.00 19.09
ATOM	1120	CB	THR			28.328	51.667	84.291	1.00 14.92
ATOM	1121	OG1	THR			28,932	52.679	85.054	1.00 18.29
ATOM	1122	CG2	THR			28.620	50.327	84.944	1.00 13.21
ATOM	1123	N	ALA			26.930	50.947	81.535	1.00 14.63
ATOM	1124	CA	ALA			26.365	49,936	80.621	1.00 17.10
ATOM	1125	C	ALA			27.213	49.686	79.354	1.00 15.52
ATOM	1126	0	ALA			27,539	48.565	79.025	1.00 16.52
ATOM	1127	CB	ALA			24.942	50.300	80.203	1.00 13.99
ATOM	1128	N	LEU			27.655	50.766	78.705	1.00 17.91
ATOM	1129	CA	LEU			28.613	50.615	77.580	1.00 17.69
ATOM	1130	C	LEU			29.895	49.851	77.846	1.00 17.03
ATOM	1131	ō	LEU			30.277	48.954	77.092	1.00 19.33
ATOM	1132	СВ	LEU			28,959	51.971	76.939	1.00 14.08
ATOM	1133	CG	LEU			27.744	52.759	76.336	1.00 14.08
ATOM	1134	CD1	LEU			27.045	52.105	75.210	1.00 11.97
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(Drawing 31)

ATOM	1135		LEU .			28.177	54.158	76.046	1.00 12.40
ATOM	1136	1/1	VAL 2			30.547	50.181	79.014	1.00 21.70
ATOM	1137	CA	VAL 2			31.713	49.315	79.310	1.00 20.85
ATOM	1138	C	VAL I			31.429	47.877	79,767	1.00 21.33
ATOM	1139	0	VAL 3	A.	160	32.086	46.918	79.409	1.00 20.74
ATOM	1140	CB	VAL 2	A	160	32.574	50.032	80.367	1.00 21.59
ATOM	1141	CGl	VAL A	A	160	33.518	49.143	81.174	1.00 20.11
ATOM	1142	CG2	VAL 3	Α	160	33.299	51.307	79.977	1.00 20.10
ATOM	1143	N	LEU A	A	161	30.351	47.711	80.542	1.00 21.86
MOTA	1144	CA	LEU A	A	161	29.850	46.366	80.838	1.00 20.50
ATOM	1145	C	LEU 2	A	161	29.462	45.490	79.613	1.00 23.16
ATOM	1146	0	LEU 1	A	161	29.753	44.294	79.564	1.00 22.89
ATOM	1147	CB	LEU I	A	161	28,623	46.472	81.733	1.00 18.65
ATOM	1148	CG	LEU 2	A	161	28.685	46.438	83.262	1.00 20.99
ATOM	1149	CD1	LEU 3	A	161	27.473	46.378	84.194	1.00 21.43
ATOM	1150	CD2	LEU I	A	161	29.729	45.492	83.855	1.00 19.30
MOTA	1151	N	ALA 2	A	162	28.755	46.146	78.648	1.00 21.20
ATOM	1152	CA	ALA A	A	162	28.384	45.496	77.361	1.00 19.14
ATOM	1153	C	ALA A	A	162	29.591	44.948	76.586	1.00 17.15
MOTA	1154	0	ALA :	A	162	29.620	43.812	76.133	1.00 20.85
ATOM	1155	ÇB	ALA 2	A	162	27.581	46.464	76.500	1.00 17.38
ATOM	1156	N	GLU 2	A	163	30.663	45.745	76.603	1.00 14.74
ATOM	1157	CA	GLU 2	A	163	31.962	45.262	76,118	1.00 17.48
MOTA	1158	С	GLU 1	A	163	32.648	44.080	76.824	1.00 22.07
ATOM	1159	0	GLU I	A	163	33.271	43.216	76.227	1.00 23.96
ATOM	1160	CB	GLU A	A	163	32.915	46.448	76.000	1.00 13.52
ATOM	1161	CG	GLU A	A.	163	34.227	46.018	75.359	1.00 13.04
ATOM	1162	CD	GLU A	A	163	35.240	47.119	75.338	1.00 15.88
ATOM	1163	OEl	GLU A		163	36.427	46.814	75.269	1.00 19.78
ATOM	1164	OE2	GLU 2			34.873	48.290	75.377	1.00 20.10
ATOM	1165	N	ILE A	A	164	32.504	44.039	78.153	1.00 20.17
ATOM	1166	CA	TLE A			32.996	42.869	78.905	1.00 18.72
ATOM	1167	C	ILE A			32.164	41.609	78.757	1.00 17.39
ATOM	1168	0	ILE 2			32.635	40.481	78.674	1.00 20.44
ATOM	1169	CB	ILE ;			33.132	43.293	80.382	1.00 20.67
ATOM	1170	CG1	ILE A			34.222	44.361	80.452	1.00 18.83
ATOM	1171	CG2	ILE A			33.398	42.110	81.345	1.00 20.70
ATOM	1172	CD1	ILE Z			34.144	45.084	81.793	1.00 21.31
ATOM	1173	N	ASN Z			30.869	41.846	78.704	1.00 18.41
ATOM	1174	CA	ASN 3			29.979	40.712	78.524	1.00 21.36
ATOM	1175	C	ASN 2			28.957	40.867	77.375	1.00 23.74
ATOM	1176	0	ASN Z			27,753	40.988	77.563	1.00 23.14
ATOM	1177	CB	ASN A			29.324	40.404	79.878	1.00 21.69
ATOM	1178	CG	ASN I			28.471	39.156	79.861	1.00 25.72
ATOM	1179		ASN 2			28.469	38.307	78.967	1.00 29.37
MOTA	1180		ASN A			27.730	39.051	80.951	1.00 27.39
MOTA	1181	N	PRO 2			29.466	40.814	76.118	1.00 25.93
ATOM	1182	CA	PRO I			28.556	40,971	74.957	1.00 26.27
ATOM	1183	C	PRO 2			27.447	39.924	74.837	1.00 24.06
ATOM	1184	ō	PRO A			26.361	40.184	74.360	1.00 24.11
ATOM	1185	ÇВ	PRO 2			29.517	41.040	73,781	1.00 25.15
ATOM	1186	CG	PRO A			30.731	40.254	74,255	1.00 27.68
ATOM	1187	CD	PRO A			30.849	40.613	75.728	1.00 24.89
ATOM	1188	N	GLN A			27.679	38.741	75.385	1.00 25.15
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(Orawing 32)

ATOM	1189	CA	GLN	A 16	7 26.552	37.804	75.508	1.00	27.09
ATOM	1190	C	GLN	A 16	7 25.275	38.321	76.189		
ATOM	1191	. Q	GLN	A 16	7 24.162	37.932	75.883		
ATOM	1192	CB	GLN	A 16'	7 27.080	36.617	76.282		
ATOM	1193	CG	GLN	A 16	7 27.386	35.380	75.468		52.12
ATOM	1194	CD	GLN	A 16			75.000		
MOTA	1195	OE	1 GLN	A 167	7 25.950		73.889		72.02
ATOM	1196	NE.	2 GLN	A 167	25.047		75.867		
ATOM	1197	N	ARG	A 168		39.242	77.156		26.04
ATOM	1198	CA	ARG .	A 168	24.389	39.900	77.837		25.63
ATOM	1199	C	ARG .	A 168	24.242	41.368	77.558		23.88
ATOM	1200	0	ARG .	A 168	23.632	42.106	78.308		24.09
ATOM	1201	CB	ARG A	A.168		39.638	79.337		24.63
ATOM	1202	CG	ARG :	A 168		38.171	79.408		25.72
ATOM	1203	CD		A 168		37.617	80.802		33.72
ATOM	1204	NE	ARG 2	A 168		38.302	81.587		35.38
ATOM	1205	CZ	ARG 2	A 168		37.877	81.680		35.91
ATOM	1206	NHI	L ARG	A 168		38.482	82.490		34.99
ATOM	1207	NH				36.867	80.975		39.28
ATOM	1208	N	GLN 2			41.810	76.424		23.22
ATOM	1209	CA	GLN A	169		43.228	76.080		19.99
ATOM	1210	C	GLN A			43.850	76.067		21.38
ATOM	1211	0	GLN A			44.969	76.492		24.60
ATOM	1212	CB	GLN A			43.510	74.769		22.07
ATOM	1213	CG	GLN A		24.970	42.878	73.494		20.93
ATOM	1214	CD	GLN A	169	25.716	43.287	72.207		23.42
ATOM	1215	OE1	GLN A	169	26.680	44.055	72.202		24.82
ATOM	1216	NE2			25.186	42.738	71.109		13.86
ATOM	1217	N	ASN A	170	22.455	43.089	75.600		20.48
ATOM	1218	CA	ASN A	170	21.138	43.736	75.525		20.97
MOTA	1219	С	ASN A	170	20.484	44.007	76.892		19.73
ATOM	1220	0	ASN A	170	19.852	45.020	77.128		18.90
ATOM	1221	CB	ASN A	170	20.195	42,930	74.595		24.43
ATOM	1222	CG	ASN A	170	20.763	42.881	73.153		25.82
ATOM	1223	ODI	ASN A	170	20.842	43.862	72.440		26.22
MOTA	1224	ND2	ASN A	170	21.197	41.709	72,734	1.00	
ATOM	1225	N	GLU A	171	20.680	43.042	77.790		21.27
ATOM	1226	CA	GLU A	171	20.166	43.146	79.157		19.84
ATOM	1227	C	GLU A	171	20.849	44,220	79.926		16.23
ATOM	1228	0	GLU A	171	20.199	45.026	80.575		20.87
ATOM	1229	CB	GLU A	171	20.317	41.822	79.892		20,47
ATOM	1230	CG	GLU A	171	19.412	40,750	79.312		24.15
ATOM	1231	CD	GLU A	171	20.157	39.825	78.376		26,37
ATOM	1232	OE1	GLU A	171	21.076	40.256	77.685		23.54
ATOM	1233	OE2	GLU A	171	19.801	38.645	78.363	1.00	
ATOM	1234	N	ILE A	172	22.169	44.236	79.751	1.00	
ATOM	1235	CA	ILE A	172	23.029	45.318	80.257	1.00	
ATOM	1236	C	ILE A	172	22.679	46.733	79.813	1.00 2	
ATOM	1237	0	ILE A	172	22.454	47.636	80.617		18.91
ATOM	1238	CB	ILE A		24.507	44.992	79.956	1.00	
ATOM	1239	CG1	ILE A		25.000	43.685	80.613	1.00	
ATOM	1240	CG2	ILE A	172	25.426	46.163	80.300		18.13
ATOM	1241	CD1	ILE A		26.426	43.320	80.163	1.00	
ATOM	1242	N	LEU A	173	22.575	46.909	78.468	1.00 2	
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[Drawing 33]

ATOM	1243	CA	LEU			22.107	48.206	77.946	1.00 17.70
ATOM	1244	С	LEU			20.699	48.622	78.396	1.00 16.26
ATOM	1245	Q	LEU			20.376	49.774	78.663	1.00 18.18
ATOM	1246	CB	LEU			22.176	48.201	76.419	1.00 16.81
ATOM	1247	CG	LEU			23.600	48.021	75.966	1.00 18.73
ATOM	1248		TEA			24.410	49.282	76.138	1.00 16.97
ATOM	1249		LEU			23.619	47.550	74.505	1.00 23.91
ATOM	1250	N	LYS			19.835	47.625	78.475	1.00 15.86
ATOM	1251	CA	LYS			18.494	47.970	78.945	1.00 19.21
ATOM	1252	С	LY\$			18.453	48.415	80.429	1.00 21.86
ATOM	1253	0	LYS			17.800	49.404	80.737	1.00 20.00
ATOM	1254	CB	LYS			17.577	46.773	78.700	1.00 20.19
ATOM	1255	CG	LYS			16.094	47.130	78.731	1.00 27.84
ATOM	1256	CD	LYS	A	174	15.799	48.368	77.858	1.00 36.14
MOTA	1257	CE	TAS			14.309	48.628	77.774	1.00 40.56
ATOM	1258	NZ	LYS	A	174	13.775	48.622	79.156	1.00 49.45
ATOM	1259	N	ARG	A	175	19.250	47.698	81.286	1.00 21.93
ATOM	1260	CA	ARG	A	175	19.476	48.145	82.686	1.00 19.65
MOTA	1261	C	ARG	A	175	20.037	49.561	82.807	1.00 16.70
MOTA	1262	0	ARG	A	175	19.476	50.419	83.468	1.00 18.12
ATOM	1263	CB	ARG	A	175	20.345	47.126	83.467	1.00 21.25
MOTA	1264	CG	ARG	A	175	20.608	47,477	84.954	1.00 22.00
MOTA	1265	CD	ARG	A	175	19.259	47.669	85.656	1.00 25.63
MOTA	1266	NE	ARG			19.435	47.923	87.095	1.00 29.77
MOTA	1267	CZ	ARG	A	175	18.601	48.672	87.834	1.00 27.58
ATOM	1268		ARG			17.550	49.301	87.355	1.00 24.74
ATOM	1269	NH2	ARG	A	175	18.792	48.780	89.073	1.00 27.45
ATOM	1270	N	GLY	A	176	21.122	49.800	82.048	1.00 17.53
MOTA	1271	CA	GLY	A	176	21.698	51.143	81.893	1.00 17.18
ATOM	1272	C	GLY			20.679	52.214	81.552	1.00 21.18
ATOM	1273	0	GLY			20.582	53.278	82.149	1.00 21.85
MOTA	1274	M	TYR			19.847	51.887	80.547	1.00 22.48
MOTA	1275	ÇA	TYR			18.756	52.787	80.117	1.00 20.51
MOTA	1276	С	TYR			17.754	53.106	81.242	1.00 18.92
ATOM	1277	0	TYR			17.406	54.246	81.542	1.00 15.84
ATOM	1278	CB	TYR			18.007	52.120	78.939	1.00 23.76
ATOM	1279	CG	TYR			17.210	53.131	78.177	1.00 24.47
MOTA	1280	ÇD1	TYR			15.817	53.306	78.397	1.00 26.61
MOTA	1281	CD2	TYR			17.941	53.869	77.236	1.00 29.40
ATOM	1282	CE1	TYR			15,139	54.308	77.661	1.00 29.08
ATOM	1283	CE2	TYR			17.270	54.855	76.515	1.00 30.70
ATOM	1284	CZ	TYR			15.899	55.092	76.747	1.00 32.01
MOTA	1285	OH	TYR			15.401	56.167	76.020	1.00 41.52
ATOM	1286	N	GLU			17.354	52.008	81.867	1.00 19.45
ATOM	1287	ÇA	GLU			16.429	52.094	82.972	1.00 22.02
ATOM	1288	С	GLU			16,820	52.802	84.236	1.00 20.09
ATOM	1289	0	GLU			16.001	53.492	84.805	1.00 21.41
MOTA	1290	CB	GLU			16.010	50.731	83.357	1.00 25.45
MOTA	1291	CG	GLU			15.173	50.032	82.303	1.00 34.73
ATOM	1292	CD	GLU			13.893	50.810	81.951	1.00 40.36
ATOM	1293		GLU			13.432	51.667	82.707	1.00 33.51
ATOM	1294	OE2	GLU			13.352	50.556	80.876	1.00 45.77
MOTA	1295	N	LEU			18.090	52.695	84.609	1.00 19.63
MOTA	1296	CA	LEU	A	179	18.655	53.567	85.665	1.00 19.10

(Drawing 34)

ATOM	1297	C	LEU .	A 179	18.366	55.051	85.511	1.00 20.25
ATOM	1298		LEU .	A 179	17.838	55.726	86.374	1.00 19.26
ATOM	1299	CB	LEU .	A 179	20.178	53.361	85.813	1.00 17.45
ATOM	1300	CG	LEU .	A 179	20.610	52.013	86.426	
ATOM	1301	CD:				51.829	87.873	
ATOM	1302	CD:	2 LEU 2	A 179	22.124	51.811	86.340	1.00 16.34
ATOM	1303	N	GLY I	A 180	18.547	55.545	84.290	1.00 19.84
ATOM	1304	CA	GLY 2	A 180	18.171	56.903	83.987	1.00 15.90
ATOM	1305	C	GLY A	A 180		57.072	84.027	1.00 17.05
ATOM	1306	0	GLY A	A 180		58.031	84.564	1.00 19.49
ATOM	1307	N	GLN A	A 181	15.923	56.068	83.478	1.00 18.19
ATOM	1308	CA	GLN A	A 181	14.448	56.207	83.522	1.00 17.92
ATOM	1309	C	GLN Z	A 181	13.822	56.265	84.938	1.00 18.20
ATOM	1310	O	GLN A	181	12.903	57.008	85.258	1.00 19.66
ATOM	1311	CB	GLN A	181		55.189	82.620	1.00 15.39
ATOM	1312	CG	GLN A	181		55.324	81.073	1.00 15.18
ATOM	1313	CD	GLN A			56.763	80.634	1.00 13.30
ATOM	1314	OE1			14.769	57.400	80.176	1.00 17.23
MOTA	1315	NE2				57.300	80.812	1.00 12.82
ATOM	1316	N	SER A		14.440	55.497	85.821	1.00 20.03
MOTA	1317	CA	SER A		14.156	55.600	87,273	1.00 16.56
ATOM	1318	c	SER A	182	14.209	56.973	87.871	1.00 14.78
ATOM	1319	0	SER A		13.305	57.369	88.582	1.00 19.49
ATOM	1320	CB	SER A	182	15.056	54.630	88.021	1.00 15.31
ATOM	1321	OG	SER A		14.563	53.332	87.714	1.00 16.70
ATOM	1322	N	ARG A	1.83	15.246	57.738	87.523	1.00 16.99
ATOM	1323	CA	ARG A		15.289	59.169	87.858	1.00 15.54
ATOM	1324	C	ARG A		14.161	60.085	87.309	1.00 19.55
ATOM	1325	0	ARG A	183	13.693	61.039	87.956	1.00 17.04
ATOM	1326	CB	ARG A		16.661	59.785	87.569	1.00 14.08
ATOM	1327	ÇG	ARG A		17.811	59.024	88.265	1.00 19.49
ATOM	1328	CD	ARG A	183	17.716	59.007	89.816	1.00 21.46
ATOM	1329	NE	ARG A	183	18.519	57.930	90.434	1.00 22.16
ATOM	1330	CŻ	ARG A	183	19.509	58.121	91.300	1.00 19.15
ATOM	1331	NH1	ARG A		19.913	59.335	91.541	1.00 16.50
ATOM	1332	NH2	ARG A	183	20.042	57.081	91.914	1.00 18.34
ATOM	1333	N	VAL A	184	13.681	59.704	86.076	1.00 21.38
ATOM	1334	CA	VAL A	184	12,494	60.417	85.589	1.00 19.02
MOTA	1335	С	VAL A	184	11.194	60.051	86.301	1.00 16.98
ATOM	1336	O	VAL A	184	10.468	60.913	86.800	1.00 18.45
ATOM	1337	CB	VAL A	1.84	12.368	60.194	84.070	1.00 19.28
ATOM	1338	CG1	VAL A	184	11.057	60.717	83.486	1.00 17.84
ATOM	1339	CG2	VAL A	184	13.517	60.605	83.169	1.00 15.69
MOTA	1340	N	ILE A	185	10.977	58.734	86.408	1.00 17.32
MOTA	1341	CA	ILE A	185	9.834	58.241	87.202	1.00 21.14
MOTA	1342	C	ILE A	185	9.790	58.797	88.672	1.00 22.77
ATOM	1343	0	ILE A	185	8,749	59.230	89.142	1.00 22.95
ATOM	1344	CB	ILE A	185	9.810	56.695	87.193	1.00 20.65
ATOM	1345	CG1	ILE A		9,555	56.195	85.758	1.00 18.43
MOTA	1346	CG2	ILE A	185	8.776	56.133	88.202	1.00 18.29
ATOM	1347	CD1	ILE A	185	9.914	54.734	85.494	1.00 14.60
ATOM	1348	N	CYS A		10.976	58.837	89.332	1.00 20.34
ATOM	1349	CA	CYS A		11.005	59.262	90.745	1.00 21.26
ATOM	1350	С	CYS A		10.979	60.766	90.931	1.00 22.80
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[Orawing 35]

ATOM	1351	O	CYS	A	186	10.775	61.304	92.009	1.00	23.98
ATOM	1352	СВ			186	12.220	58.669	91.429		21.60
MOTA	1353	SG			186	12.075	58.576	93.237		25.16
ATOM	1354	N	GLY	A	187	11.149	61.494	89.814		20.34
ATOM	1355	CA	GLY	A	187	11.023	62.949	89.981	1.00	13.75
MOTA	1356	С	GLY	Α	187	12,351	63.676	90.197	1.00	14.55
ATOM	1357	0	GLY	Α	187	12.436	64.871	90.453	1.00	16.08
ATOM	1358	N	TYR	. A	188	13.434	62.894	90.072	1.00	14.86
ATOM	1359	CA	TYR	A	188	14.736	63.526	90.226	1.00	16.85
ATOM	1360	C	TYR	A	188	15.214	64.366	89.046	1.00	20.24
MOTA	1361	0	TYR	A	188	15.979	65.318	89.192	1.00	18.42
ATOM	1362	CB	TYR	A	188	15.758	62.441	90.479	1.00	21.64
ATOM	1363	CG	TYR	A	188	15.683	61.913	91.899	1.00	27.20
ATOM	1364	CD1	TYR	A	188	16.748	62.226	92.737	1.00	32.00
ATOM	1365	CD2	TYR	A	188	14.604	61.128	92.365	1.00	30.81
ATOM	1366	CEL	TYR	A	188	16.727	61.784	94.071	1.00	35.38
ATOM	1367	CE2	TYR	A	188	14.570	60.695	93.705	1.00	30.65
ATOM	1368	CZ	TYR	A	188	15.624	61.071	94.556	1.00	35.88
ATOM	1369	OH	TYR	A	188	15.607	60.787	95.912		41.39
MOTA	1370	N	HIS	A	189	14.751	63.890	87,862	1.00	19.35
ATOM	1371	CA	HIS	A	189	15.215	64.475	86.589	1.00	19.83
ATOM	1372	Ç	HIS	A	189	14.108	64.550	85.551	1.00	16.85
ATOM	1373	0	HIS	A	189	13.232	63.689	85.533	1.00	18.10
ATOM	1374	CB	HIS	A	189	16.360	63.648	86.032	1.00	15.43
MOTA	1375	CG	HIS	A	189	17.677	64.142	86.589	1.00	16.05
ATOM	1376	ND1	HIS	A	189	18.154	65.384	86.414		17.24
ATOM	1377	CD2	HIS	A	189	18,581	63.433	87.382		17.58
ATOM	1378	CEL	HIS	A	189	19.347	65.503	87.080	1.00	16.38
ATOM	1379	NE2	HIS	A	189	19.587	64.304	87.667	1.00	18.68
ATOM	1380	N	TRP	A	190	14.183	65.611	84.723	1.00	18.99
MOTA	1381	CA	TRP	A	190	13.341	65.719	83.490	1.00	17.22
ATOM	1382	C	TRP	A	1.90	13,768	64.690	82,453	1.00	15.79
ATOM	1383	0	TRP	A	190	14,942	64.354	82.416	1.00	15.77
ATOM	1384	CB	TRP	A	190	13.502	67.102	82.855	1.00	16.12
ATOM	1385	ĊĠ	TRP	A	190	13.134	68.178	83,857	1.00	12.39
MOTA	1386	CD1	TRP	A	190	14.033	69.073	84.454	1.00	10.88
MOTA	1387	CD2	TRP	A	190	11.800	68.581	84.295		14.35
ATOM	1388	NE1	TRP	A	190	13.343	69.989	85.186	1.00	13.77
MOTA	1389	CE2	TRP	A	190	11.976	69.731	85.141	1.00	10.71
ATOM	1390	CE3	TRP	A	190	10.505	68.036	84.094	1.00	14.68
MOTA	1391	CZ2	TRP	A	190	10.845	70.401	85.666	1.00	12.89
ATOM	1392	CZ3	TRP	A	190	9.393	68.700	84.672	1.00	16.93
ATOM	1393	CH2	TRP	A	190	9.557	69.875	85.441	1.00	12.82
MOTA	1394	И	GLN	A	191	12,859	64.188	81.613	1.00	16.92
ATOM	1395	CA	GLN	A	191	13.316	63.234	80.569	1.00	17.49
ATOM	1396	C	GLN	A	191	14,519	63.720	79.696	1.00	15.64
MOTA	1397	0	GLN	A	191	15.508	63.054	79.429	1.00	16.52
MOTA	1398	CB	GLN			12.113	62.829	79.721	1.00	15.28
ATOM	1399	CG	GLN	A	191	12.522	61.832	78.632	1.00	17.26
ATOM	1400	CD	GLN			12.860	60.493	79.206	1.00	16.84
ATOM	1401	ÖE1	GLN	A	191	12.086	59.920	79.946	1.00	21.21
ATOM	1402	NE2	GLN	A	191	14.027	59.971	78.864	1.00	16.69
ATOM	1403	N	SER	A	192	14.448	65.016	79.389	1.00	
ATOM	1404	CA	SER	A	192	15.564	65.622	78.666		15.81

[Drawing 36]

MOTA	1405	С	SER	A	192	16.899	65.683	79,339	1.00	17,61
MOTA	1406	0	SER			17.937	65.640	78.700		16.65
MOTA	1407	CB	SER			15.209	67.005	78.211		15.87
ATOM	1408	OG	SER			14.862	67.833	79.313	1.00	
ATOM	1409	N	ASP			16.886	65.712	80.681		17.44
ATOM	1410	CA	ASP			18,190	65.648	81.360		15.23
ATOM	1411	C	ASP	A	193	18.889	64.316	81.197	1.00	12.26
ATOM	1412	٥	ASP	A	193	20.113	64.204	81.054	1.00	
ATOM	1413	CB	ASP	A	193	18.036	65.832	82.887	1.00	
ATOM	1414	CG	ASP	A	193	17.367	67.116	83.311	1.00	
MOTA	1415		ASP			17.503	68.180	82.691	1.00	15.89
atom	1416	OD2	ASP			16.673	67.038	84.312	1.00	
ATOM	1417	N	VAL	A	194	18.025	63.283	81,216	1.00	11.48
ATOM	1418	ÇA	VAL	A	194	18.425	61.882	81.108	1.00	
ATOM	1419	С	VAL	A	194	18.851	61.478	79.663	1.00	15.63
ATOM	1420	0	VAL	A	194	19.852	60.822	79.387	1.00	15.79
ATOM	1421	CB	VAL	A	194	17.210	61.135	81.625	1.00	16.33
MOTA	1422	CG1	VAL	A	194	17.152	61.202	83.184	1.00	19.26
MOTA	1423	CG2	VAL	A	194	17.079	59.712	81.106	1.00	17.70
ATOM	1424	N	ASP	A	195	18.050	61.992	78.728	1.00	16.54
ATOM	1425	CA	ASP	A	195	18.488	61.921	77.332	1.00	16.55
ATOM	1426	C	ASP	A	195	19.801	62.636	77.029	1.00	14.74
ATOM	1427	0	ASP	A	195	20.758	62.076	76.519	1.00	18.92
ATOM	1428	CB	ASP	A	195	17.367	62.455	76.477	1.00	15.91
MOTA	1429	CG	ASP	A	195	16.139	61.563	76.560	1.00	19.86
MOTA	1430	QD1	ASP	A	195	16.153	60.385	76.922	1.00	27.62
ATOM	1431	QD2	ASP	A	195	15.090	62.069	76.264	1.00	26.20
ATOM	1432	N	ALA	A	196	19.902	63.900	77.450	1.00	15.85
ATOM	1433	CA	ALA	Α	196	21.203	64.555	77.312	1.00	14.73
ATOM	1434	C	ALA	A	196	22.383	63.806	77.932	1.00	18.85
ATOM	1435	0	ALA	A	196	23.512	63.751	77.429	1.00	21.06
ATOM	1436	ĊВ	ALA	A	196	21.134	65.950	77.904	1.00	13.59
ATOM	1437	N	ALA	A	197	22.056	63.177	79.091	1.00	19.14
ATOM	1438	CA	ALA	A	197	23.098	62.442	79.808	1.00	17.62
ATOM	1439	C	ALA	Α	197	23.644	61.202	79.090	1.00	17.71
ATOM	1440	0	ALA	A	197	24.851	60.931	79.104	1.00	18.18
MOTA	1441	CB	ALA	A	197	22.587	62.002	81.181	1.00	15.91
ATOM	1442	N	ARG	Ą	198	22,711	60.477	78.418	1.00	16.04
atom	1443	CA	ARG	A	198	23.238	59.409	77.565	1.00	15.41
MOTA	1444	C	ARG	A	198	24.179	59.843	76.413	1.00	14.99
ATOM	1445	0	ARG	A	198	25.194	59.219	76.113	1.00	17.07
MOTA	1446	CB	ARG	A	198	22,136	58.469	77.080	1.00	14.50
MOTA	1447	CG	ARG	A	198	21.195	58.043	78.179	1.00	16.67
ATOM	1448	CD	ARG	A	198	20.142	57.044	77.730	1.00	19.20
ATOM	1449	NE	ARG	A	198	19.280	56.629	78.849	1.00	22.72
ATOM	1450	CZ	ARG	A	198	18.003	57.012	79.061	1.00	22.30
ATOM	1451	NH1	ARG	A	198	17.412	57.905	78.325	1.00	20.95
MOTA	1452	NH2	ARG .	A	198	17.292	56.518	80.045	1.00	21.57
ATOM	1453	N	VAL	A	199	23.907	61.030	75.842	1.00	17.69
ATOM	1454	CA	VAL .	A	199	24.961	61.510	74.913	1.00	17.44
MOTA	1455	С	VAL			26.376	61.736	75.457	1.00	18.48
MOTA	1456	0	VAL			27.360	61.145	74.988	1.00	19.25
atom	1457	CB	VAL	A	199	24.452	62.820	74.284	1.00	15.93
ATOM	1458	CG1	VAL .	A	199	25.350	63.540	73.279	1.00	11.45

[Drawing 37]

ATOM	1459	CG2	VAL A	Ą	199	23.072	62.640	73.680	1.00 15.33
ATOM	1460	N	VAL 2			26.471	62.548	76.550	1.00 18.52
ATOM	1461	CA	VAL 2	A .	200	27.822	62.723	77.153	1.00 16.27
MOTA	1462	C	VAL A	Α.	200	28.442	61.461	77.794	1.00 14.37
ATOM	1463	0	VAL 2	A .	200	29.643	61.247	77.797	1.00 17.25
ATOM	1464	CB	VAL A	1	200	27.811	63.938	78.100	1.00 16.56
MOTA	1465	CG1	VAL A	1	200	26.761	63.894	79.246	1.00 13.52
ATOM	1466	CG2	VAL A	.	200	27.666	65.177	77.226	1.00 17.14
ATOM	1467	N	GLY A	,	201	27.556	60.570	78.283	1.00 14.98
ATOM	1468	CA	GLY A	1	201	27.998	59.297	78.836	1.00 13.20
MOTA	1469	С	GLY A	١.	201	28.609	58.377	77.824	1.00 16.81
ATOM	1470	0	GLY A	1 :	201	29.588	57.701	78.067	1.00 17.03
ATOM	1471	N	SER A	A :	202	28.034	58.413	76.614	1.00 17.82
ATOM	1472	CA	SER A	. .	202	28.757	57.750	75.509	1.00 16.70
ATOM	1473	С	SER A	A :	202	30.087	58.390	75.104	1.00 13.91
ATOM	1474	0	SER A	١.	202	31,117	57.765	74.939	1.00 18.47
ATOM	1475	CB	SER A	. :	202	27.813	57.511	74.303	1.00 15.94
ATOM	1476	OG	SER A			27.634	58.737	73.600	1.00 19.26
MOTA	1477	N	ALA A	٠.	203	30.094	59.719	75.030	1.00 14.76
ATOM	1478	CA	ALA A			31.333	60.383	74.641	1.00 14.41
ATOM	1479	C	ALA A			32.527	60.110	75.563	1.00 17.92
ATOM	1480	0	ALA A			33.652	59.803	75.177	1.00 16.36
ATOM	1481	ÇВ	ALA A			31.042	61.872	74.572	1.00 13.61
ATOM	1482	N	VAL A			32,200	60.126	76.880	1,00 18.27
ATOM	1483	CA	VAL A			33.290	59.835	77.823	1.00 16.45
ATOM	1484	C	VAL A			33,834	58.394	77.718	1.00 13.88
ATOM	1485	0	VAL A			35.015	58.177	77.916	1.00 17.64
ATOM	1486	СВ	VAL A			32.893	60.245	79.285	1.00 16.63
ATOM	1487	CG1	VAL A			34.131	60.323	80.177	1.00 16.51
ATOM	1488	CG2	VAL A			31.836	59.301	79.862	1.00 13.53
ATOM	1489	N	VAL A			32.971	57.392	77.355	1.00 15.31
ATOM	1490	CA	VAL A			33.648	56.100	77.152	1.00 17.30
ATOM	1491	C	VAL A			34.636	56.018	75.953	1.00 19.20
ATOM	1492	0	VAL A			35.644	55.311	76.002	1.00 19.08
ATOM	1493	CB	VAL A			32.563	55.014	77.035	1.00 19.10
ATOM	1494		VAL A			32.812	53,549	76.681	1.00 16.51
ATOM	1495	CG2				31.797	55.088	78.343	1.00 17.27
ATOM	1496	N	ALA A			34.409	56.893	74.934	1.00 21.03
ATOM	1497	CA	ALA A			35.452	57.018	73.888	1.00 19.17
ATOM	1498	C	ALA A			36.765	57,498	74.469	1.00 19.27
ATOM	1499	ō	ALA A			37.809	56.868	74.353	1.00 18.59
ATOM	1500	СВ	ALA A			34.982	57.988	72.809	1.00 16.60
ATOM	1501	N	THR A			36.643	58.597	75.234	1.00 20.20
ATOM	1502	CA	THR A			37.873	59.078	75.903	1.00 20.52
ATOM	1503	C	THR A			38.613	58.126	76,838	1.00 21.84
ATOM	1504	ő	THR A			39.831	58.030	76.898	1.00 23.45
ATOM	1505	ĊВ	THR A			37.659	60.341	76.674	1.00 19.84
ATOM	1506	OG1	THR A			36.577	61.100	76.137	1.00 20,11
ATOM	1507	CG2	THR A			38.945	61.141	76.710	1.00 20.11
ATOM	1508	И	LEU A			37.806	57.345	77.574	1.00 23.89
ATOM	1509	CA	LEU A			38.323	56.332	78.510	1.00 22.19
ATOM	1510	C	LEU A			39.165	55.285	77.783	1.00 25.28
ATOM	1511	ō	LEU A			40.232	54.880	78.228	
ATOM	1512	СВ	LEU A			37.170	55.700	79.312	1.00 24.41 1.00 21.93
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(Drawing 38)

ATOM	1513	CG		A	208	36,238	56.370	80.325	1.00	24.99
atom	1514	CD1			208	35.117	55.631	81.058	1.00	26.55
ATOM	1515	CD2			208	37.303	56.685	81.377		22.67
ATOM	1516	N			209	38.666	54.873	76.588		24.64
ATOM	1517	CA	HIS	A	209	39.495	53.970	75.766	1.00	20.81
ATOM	1518	C	HIS	A	209	40.863	54.530	75.277	1.00	19.15
ATOM	1519	0	HIS	A	209	41.807	53.872	74.934	1.00	20.30
ATOM	1520	CB			209	38.656	53.449	74.595	1.00	16.87
ATOM	1521	CG	HIS	Α	209	37.588	52.476	74.994	1.00	13.42
MOTA	1522	ND1	HIS	Α	209	36.335	52.782	75.375	1.00	14.12
MOTA	1523	CD2	HIS	A	209	37.686	51.099	74,975	1.00	12.37
MOTA	1524	CE1	HIS	A	209	35.653	51.616	75.586	1.00	10.36
ATOM	1525	NE2	HIS	A	209	36.493	50.590	75.334	1.00	13.53
ATOM	1526	N	THR	A	210	41.035	55.827	75.336	1.00	20.82
ATOM	1527	CA	THR	A	210	42.393	56.384	75.116	1.00	21.40
ATOM	1528	C	THR	A	21.0	43,396	56.387	76.298	1.00	26.34
ATOM	1529	0	THR	A	210	44.567	56.745	76.188	1.00	27.83
ATOM	1530	CB	THR	A	210	42.315	57.836	74.662	1.00	22.02
ATOM	1531	OG1	THR	A	210	42.096	58.723	75.795	1.00	25.04
ATOM	1532	CG2	THR	A	210	41.307	58.070	73.528		20.46
ATOM	1533	N	ASN	A	211	42.844	56.032	77,482	1.00	26.32
ATOM	1534	CA	ASN	A	211	43.544	56.135	78.782	1.00	23.64
ATOM	1535	С	ASN	A	211	44.212	54,824	79.204		20.62
ATOM	1536	0	ASN	A	211	43.591	53.785	79.374	1.00	22.28
ATOM	1537	CB	ASN	A	211	42.563	56.749	79.827	1.00	24.99
MOTA	1538	CG	ASN	A	211	43.226	56.863	81.206		24.09
ATOM	1539	OD1	ASN			43.320	55.886	81.935		25.25
ATOM	1540	ND2	ASN	A	211	43.689	58,038	81.566		21.24
ATOM	1541	N	PRO	A	212	45.563	54.884	79.353		22.01
MOTA	1542	CA	PRO	A	212	46.337	53.660	79.633	1.00	21.52
ATOM	1543	С	PRO	A	212	45.859	52.883	80.848	1.00	22.52
MOTA	1544	0	PRO	Α	212	45.670	51.673	80.882		22.12
MOTA	1545	CB	PRO	A	212	47.743	54.190	79.845	1.00	22.52
ATOM	1546	CG	PRO	A	212	47.805	55.535	79.117	1.00	25.40
ATOM	1547	CD	PRO	A	212	46.391	56.076	79.175	1.00	22.01
ATOM	1548	И	ALA	A	213	45.626	53.674	81.897		23.96
ATOM	1549	ÇA	ALA	A	213	45.139	53.025	83.140	1.00	23.57
MOTA	1550	C	ALA	A	213	43.797	52.337	83.019	1.00	21.76
ATOM	1551	0	ALA	A	213	43.600	51.185	83.403	1.00	24.96
ATOM	1552	CB	ALA	A	213	45.039	54.071	84.259	1,00	21.55
ATOM	1553	N	PHE	Α	214	42.885	53.085	82.373		19.30
ATOM	1554	CA	PHE	A	214	41.617	52.431	82.017	1.00	20.02
ATOM	1555	C	PHE	A	214	41.798	51.170	81.197		20.38
MOTA	1556	0	PHE	A	214	41.255	50.120	81.510		18.82
ATOM	1557	CB	PHE	A	214	40.690	53.445	81.314		23.58
ATOM	1558	CG	PHE			39.367	52.839	80.840		26.35
ATOM	1559	CDl	PHE	A	214	38.249	52.765	81.711		24.66
ATOM	1560	CD2	PHE	A	214	39.262	52.365	79.507		23.08
ATOM	1561		PHE			37.032	52.191	81.265		27.05
ATOM	1562		PHE			38.052	51.792	79.077	1.00	
ATOM	1563	CZ	PHE			36.951	51.697	79.944	1.00	
ATOM	1564	N	GLN			42.654	51.298	80.149	1.00	
ATOM	1565	CA	GLN			42,904	50.137	79.275	1.00	
ATOM	1566	С	GLN			43.354	48.889	79.995	1.00	
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(Drowing 39)

ATOM	1567	٥	GLN	A	215	42.823	47.783	79.875	1.00 21.17
MOTA	1568	CB			215	43.970	50.520	78.278	1.00 22.37
MOTA	1569	CG			215	43.483	51.517	77.261	1.00 22.30
ATOM	1570	CD			215	44.662	52.026	76.479	1.00 28.18
MOTA	1571	OE1			215	45.821	51.695	76.664	1.00 32.27
ATOM	1572	NE2			215	44.359	52.947	75.605	1.00 28.35
ATOM	1573	И	GLN	A	216	44.375	49.154	80.845	1.00 24.60
MOTA	1574	CA			216	44.876	48.089	81.718	1.00 25.81
MOTA	1575	C	GLN	A	216	43.909	47.530	82.715	1.00 23.07
ATOM	1576	O	GLN	A	216	43.822	46.328	82.899	1.00 22.91
MOTA	1577	CB	GLN	A	216	46.052	48.544	82.517	1.00 33.78
MOTA	1578	CG	GLN	A	216	47.181	49.037	81.631	1.00 49.94
ATOM	1579	CD	GLN	A	216	48,161	49.693	82.574	1.00 61.57
ATOM	1580	OE1	GLN	A	216	48.354	49.243	83.704	1.00 69.34
ATOM	1581	NE2	GLN	Α	216	48.737	50.805	82.114	1.00 63.50
ATOM	1582	N	GLN	A	217	43.155	48.439	83.377	1.00 22.58
MOTA	1583	CA	GLN	A	217	42.099	47.917	84.261	1.00 23.69
ATOM	1584	C	GLN	A	217	40.971	47.113	83.590	1.00 24.92
ATOM	1585	0	GLN	A	217	40.480	46.102	84.088	1.00 24.09
ATOM	1586	ÇB	GLN	A	217	41.565	49.042	85.189	1.00 23.44
ATOM	1587	CG	GLN	A	217	40.720	48.541	86.407	1.00 23.24
ATOM	1588	CD	GLN	A	217	41.489	47.589	87.335	1.00 21.58
ATOM	1589	OE1	GLN	A	217	42.676	47.749	87.598	1.00 24.82
ATOM	1590	NE2	GLN	A	217	40.827	46.516	87.744	1.00 19.85
MOTA	1591	N	LEU	A	218	40.628	47.595	82.390	1.00 26.00
MOTA	1592	CA	LEU	A	218	39.701	46.859	81.532	1.00 23.47
ATOM	1593	C	LEU	A	218	40.195	45.495	81.093	1.00 21.96
MOTA	1594	0	LEU	Α	218	39.476	44.515	81.209	1.00 22.51
MOTA	1595	CB	LEU	A	218	39.309	47.724	80.327	1.00 23.65
MOTA	1596	CG	LEU	A	218	38.292	47.073	79.369	1.00 21.04
MOTA	1597	CD1	LEU	A	218	38.103	47.980	78.168	1.00 25.30
MOTA	1598	CD2	LEU	A	218	36,952	46.736	80.004	1.00 13.22
MOTA	1599	N	GLN	A	219	41.451	45.425	80.640	1.00 23.49
ATOM	1600	CA	GLN	A	219	42.033	44.079	80.457	1.00 29.37
MOTA	1601	С	GLN	A	219	41.880	43.156	81.681	1.00 29.60
ATOM	1602	0	GLN	A	219	41.455	42.016	81.569	1.00 29.63
ATOM	1603	CB	GLN	A	219	43.544	44.131	80.199	1.00 37.46
ATOM	1604	CG	GLN	A	219	44.052	44.703	78.867	1,00 51.74
ATOM	1605	CD	GLN	A	219	45.511	45.267	78.911	1.00 60.29
MOTA	1606	OE1	GLN	A	219	46.415	44.774	79.568	1.00 65.45
ATOM	1607	NE2	GLN	A	219	45.764	46.352	78.161	1.00 60.81
ATÓM	1608	N	LYS	A	220	42.206	43.722	82.879	1.00 28.28
ATOM	1609	CA	LYS	A	220	42.004	42.926	84.111	1.00 26.68
ATOM	1610	С	LYS	A	220	40.588	42.446	84.386	1.00 24.54
MOTA	1611	Q	LYS	A	220	40.347	41.275	84.640	1.00 26.87
MOTA	1612	CB	LYS	A	220	42.591	43.631	85.319	1.00 29.93
ATOM	1613	CG	LYS	A	220	44.019	43.952	84.934	1.00 36.96
ATOM	1614	CD	LYS			45.015	44.044	86.081	1.00 47.54
ATOM	1615	CE	LYS	A	220	44.741	45.121	87,121	1.00 55.28
ATOM	1616	NZ	LYS	A	220	44.868	46.456	86.510	1.00 61.92
ATOM	1617	N	ALA	A	221	39.630	43.379	84.217	1.00 21.04
ATOM	1618	CA	ALA	A	221	38.215	42.960	84.307	1.00 18.69
MOTA	1619	C	ALA	A	221	37.761	41.903	83.291	1.00 24.31
MOTA	1620	0	ALA	A	221	37.095	40.921	83.598	1.00 26.78

[Drawing 40]

ATOM	1621		ALA	. A	221	37.306	44,177	84.140	1.00	14.85
atom	1622		LYS	7	222	38,223	42.106	82,029	1.00	24.15
ATOM	1623	CA	LYS	. 2	. 222	38.065	41.018	81.046		23.96
MOTA	1624	C	LYS	A	. 222	38.668	39.675	B1.431		22.61
ATOM	1625	0	LYS	A	. 222	38.023	38.628	81.422		21.88
ATOM	1626	CB	LYS	A	222	38.591	41,444	79.659		22.92
MOTA	1627	CG	LYS	A	222	37.682	42.516	79.109		22.76
ATOM	1628	CD	LYS	A	222	38.038	42.903	77.691		22.50
ATOM	1629	ÇE	LYS	A	222	37.050	43.918	77.109		22.07
ATOM	1630	NZ	LYS	A	222	37.556	44.613	75.909		21.47
MOTA	1631	N	ALA	A	223	39.949	39.728	81.830		22.84
ATOM	1632	CA	ALA	A	223	40.533	38.472	82.353		25.17
ATOM	1633	C	ALA	A	223	39.812	37.842	83.543		26.19
ATOM	1634	0	ALA	A	223	39.534	36.652	83.573		27.99
ATOM	1635	CB	ALA	A	223	42.013	38.638	82.691		21.36
ATOM	1636	N	GLU	A	224	39.424	38.696	84.487		27.72
ATOM	1637	ÇA	GLU	A	224	38.643	38.187	85.610		27.59
ATOM	1638	C	GLU	A	224	37.338	37.525	85.191		28.89
MOTA	1639	0	GLU	A	224	36,971	36.412	85.568		28.75
ATOM	1640	CB	GLU	A	224	38.476	39.348	86.606		29.18
ATOM	1641	CG	GLU	A	224	37.470	39.099	87.741		29.05
ATOM	1642	CD	GLU	A	224	37.335	40.348	88,557		29.41
MOLY	1643	OE1	GLU	A	224	36.506	41.189	88.269		28.08
ATOM	1644	OE2	GLU	A	224	38.060	40.487	89.516		31.14
ATOM	1645	N	PHE	A	225	36.659	38.233	84.288		29.33
ATOM	1646	ÇA	PHE	A	225	35.398	37.699	83.770		28.70
ATOM	1647	С	PHE			35.551	36.354	83.061		31.53
ATOM	1648	0	PHE			34.802	35.399	83.222		29.17
MOTA	1649	CB	PHE	A	225	34.786	38.756	82.858		26.48
MOTA	1650	CG	PHE	A	225	33.449	38.281	82.361		25.90
MOTA	1651	CD1	PHE	Α	225	32.361	38,258	83,250		28.71
ATOM	1652	CD2	PHE	A	225	33.317	37.854	81.022		28.64
ATOM	1653	CE1	PHE	A	225	31,129	37.758	82.817		29.98
MOTA	1654	CE2	PHE	A	225	32,074	37.365	80.564		28.53
ATOM	1655	CZ	PHE	A	225	30.998	37.309	81.479	1.00	30.02
ATOM	1656	N	ALA	A	226	36,635	36.308	82,289		31.91
MOTA	1657	CA	ALA	A	226	36.996	35.055	81.643	1.00	36.17
ATOM	1658	C	ALA	Α	226	37.178	33.829	82.536		40.94
ATOM	1659	0	ALA	A	226	36.704	32.735	82.271		41.75
ATOM	1660	ÇВ	ALA	A	226	38.284	35,261	80.877		33.81
MOTA	1661	N	GLN	A	227	37.883	34.081	83.647		44.91
ATOM	1662	CA	GLN	A	227	38.067	32.931	84.543	1.00	49.32
ATOM	1663	C	GLN	A	227	36.782	32.579	85.233	1.00	51.62
ATOM	1664	0	GLN .	A	227	36.396	31.467	85.528		
ATOM	1665	CB	GLN .	A.	227	39.239	33.150	85.499		50.80
ATOM	1666	CG	GLN .			40.441	33.937	84.900		59.92
MOTA	1667	ÇD	GLN .	A	227	40.812	33,681	83.397		68.68
ATOM	1668	QE1	GLN .	Ä	227	40.799	32.592	82.834		73.97
ATOM	1669	NE2	GLN :			41,221	34.764	82.726	1.00	
ATOM	1670	N	HIS ?			36.041	33.669	85.411	1.00	
MOTA	1671	ÇA	HIS !	A	228	34.687	33.501	85.925	1.00	
ATOM	1672	C	HIS .	A	228	33,816	32.519	85.143	1.00	
MOTA	1673	0	HIS 2	A	228	33.014	31.764	85.662	1.00	
ATOM	1674	CB	HIS I	A.	228	34.091	34.903	86.045	1.00	

[Drawing 41]

MOTA	1675	ÇG			228	32.632	34.780	86.283	1.00 71.87
ATOM	1676	ND1			228	31.729	34.968	85.313	1.00 74.51
ATOM	1677	•	HIS			32.007	34.363	87.458	1.00 75.53
MOTA	1678	CE1			228	30.511	34.660	85.852	1.00 78.68
MOTA	1679	NE2			228	30.682	34.288	87.171	1.00 80.02
MOTA	1680	N			229	34.061	32.545	83.846	1.00 74.45
MOTA	1681	CA			229	33.306	31.659	82.963	1.00 81.06
MOTA	1682	С			229	33.569	30.149	83.028	1.00 85.59
ATOM	1683	0			229	33.123	29.427	82.135	1.00 86.52
MOTA	1684	CB			229	33.477	32.181	81.530	1.00 81.47
MOTA	1685	CG			229	33.002	33.631	81.335	1.00 81.55
ATOM	1686	CD	GLN	A	229	31.488	33.690	81.352	1.00 83.81
MOTA	1687	OE1			229	30.804	33.832	82.355	1.00 82.35
ATOM	1688	NE2			229	30.950	33.588	80.141	1.00 86.80
MOTA	1689	N	LYS	Α	230	34.317	29.749	84.086	1.00 91.08
ATOM	1690	CA	LYS	A	230	34.965	28.444	84,325	1.00 95.45
ATOM	1691	CB	LYS	A	230	33.976	27.245	84.052	1.00 97.51
ATOM	1692	CG	LYS	A	230	34.256	26.053	83.073	1.00 9B.30
MOTA	1693	CD	LYS	A	230	34.035	26.121	81.534	1.00 98.30
ATOM	1694	CE	LYS	A	230	34.810	27.172	80.713	1.00100.00
MOTA	1695	NZ	LYS	A	230	36.244	27,239	81.067	1.00100.00
ATOM	1696	C	LYS	A	230	36.409	28.279	83.743	1.00 97.06
ATOM	1697	OCT1	LYS	A	230	36.876	29,108	82.942	1.00 95.86
MOTA	1698	OCT2	LYS	A	230	37.052	27.241	83.957	1.00 99.89
ATOM	1935	s	504	ន	231	22.561	63.872	89.148	1.00 45.29
ATOM	1936	01	504	S	231	21.748	62.858	88.279	1.00 50.45
MOTA	1937	02	SO4	S	231	21.648	64.707	90,036	1.00 51.74
ATOM	1938	03	SO4	S	231	23.551	63.095	90.035	1.00 49.75
ATOM	1939	04	SO4	S	231	23.260	64.912	88.285	1.00 44.08
MOTA	1	0	нон	W	232	10.522	63.513	85.670	1.00 17.86
MOTA	2	0	HOH	W	233	34.116	63.633	80.578	1.00 20.45
MOTA	3	0	HOH	M	234	7.928	61.775	88.229	1.00 15.62
MOTA	4	0			235	10.374	64.545	82.597	1.00 14.58
ATOM	5	0	HOH			15.375	75.641	85.508	1.00 22.07
MOTA	6	0	нон	M	237	20.773	44.507	86.785	1.00 18.67
MOTA	7	0	нон	M	238	32,701	49.912	75.935	1.00 15.79
MOTA	8	0	нон	W	239	21.979	72.096	84.493	1.00 19.08
ATOM	9	0	нон	W	240	13.158	73.905	82.705	1.00 27.34
ATOM	10	0	нон	W	241	14.358	71.880	73.410	1.00 26.83
ATOM	11	0	нон	W	242	5.537	80.043	74.802	1.00 23.33
MOTA	12	0	нон	W	243	36.136	62.604	78.407	1.00 23.19
ATOM	13	O	HOH	W	244	30.393	53.028	87.579	1.00 19.02
MOTA	14	O			245	28.532	49.107	93.252	1,00 21.32
MOTA	15	0	нон			24.657	73.146	75.882	1.00 20.92
ATOM	16	0	HOH			10.080	55.567	81.848	
MOTA	17	O	HOH			29.907	52,840	73.379	1.00 22.59
MOTA	18	0	нон			38.583	48.054	74.575	1.00 24.10
ATOM	19	0	нон			29.465	68.020	86.676	1.00 32.30
ATOM	20	O	нон			12.847	73.680	85.460	1.00 40.76
ATOM	21	O	нон			5.516	59.770	95.129	1.00 40.84
MOTA	22	0	нон			42.504	47.354	77.319	1.00 30.77
ATOM	23	0	нон			13.495	75.378	74.412	1.00 22.57
ATOM	24	O	нон			17.100	76.564	77.737	1,00 30.00
MOTA	25	0	нон	M	256	33.508	40.103	102.712	1.00 26.49

[Drawing 42]

ATOM	26	0	HOH W	257	20.825	55.648	81.278	1.00 20.11
ATOM	27	O	нон и	258	19.730	61,701	89.970	1.00 23.10
ATOM	28	0	HOH W		4.363	74.520	80.720	1.00 33.74
ATOM	29	0	HOH W	260	31.490	42.656	98.480	1.00 34.19
MOTA	30	0	HOH W	261	6.696	75.130	78.477	1.00 15.66
ATOM	31	0	нон w	262	10.667	67.023	75.103	1.00 38.86
MOTA	32	0	HOH W	263	8.252	64.433	92.307	1.00 23.15
ATOM	33	0	HOH W	264	41.924	51.223	74.247	1.00 30.19
ATOM	34	0	HOH M	265	1.437	67.705	89.398	1.00 39.48
ATOM	35	Q	HOH W	266	4.055	66.946	91.467	1.00 29.22
ATOM	36	٥	нон м	267	3.092	69.112	84.950	1.00 25.58
ATOM	37	0	HOH W	268	9.537	59.065	79.795	1.00 30.90
ATOM	38.	0	нон м	269	9.306	83.197	79.638	1.00 44.19
ATOM	39	0	HOH W	270	34.786	41.166	75.522	1.00 32.98
ATOM	40	0	HOH W		28.084	37.193	84.163	1.00 30.43
ATOM	41	0	HOH W	272	40.742	49.227	76.024	1.00 21.82
MOTA	42	0	HOH W	273	35.074	40.712	85.668	1.00 29.87
ATOM	43	0	M HOH	274	30.318	45,526	96.384	1.00 35.57
MOTA	44	O	HOH W	275	31.493	69.162	80.850	1.00 19.51
MOTA	45	0	HOH W	276	42.914	61.700	76.016	1.00 28.69
ATOM	46	0	HOH W	277	34.422	64.714	92.625	1.00 38.81
MOTA	47	0	HOH M	278	13.405	78.374	80.916	1.00 25.22
ATOM	48	0	HOH W	279	44.634	57.811	84.433	1.00 31.73
ATOM	49	0	HOH W		44.303	60.992	82.740	1.00 28.14
MOTA	50	O	HOH W	281	32.596	51.432	73.247	1.00 22.63
ATOM	51	0	HOH W	282	22.182	40.126	75.125	1.00 27.50
ATOM	52	0	HOH W	283	18.482	55.362	89.100	1.00 21.25
MOTA	53	0	и нон	284	36.960	42.360	74.192	1.00 28.88
MOTA	54	0	HOH W	285	35.881	48.845	94.047	1.00 26.90
ATOM	55	0	HOH W	286	26.212	59.698	94,760	1.00 23.37
ATOM	56	0	HOH W	287	29.246	44.303	73.369	1.00 40.38
ATOM	57	O	HOH W	288	27,356	35.947	80,422	1.00 31.74
MOTA	58	0	HOH W	289	40.482	45.029	76.766	1.00 30.88
ATOM	59	0	HOH W	290	24.864	58.724	91.112	1.00 25.30
MOTA	60	0	нон м	291	28.560	61.547	91.755	1.00 39.37
MOTA	61	0	HOH M	292	27.888	63.113	90.252	1.00 40.28
ATOM	62	0	HOH W	293	31.069	41.023	103.435	1.00 38.13
ATOM	63	0	HOH M		5.144	47.860	86.978	1.00 37.63
ATOM	64	O	HOH M	295	29.373	52.425	90.409	1.00 21.69
MOTA	65	0	HOH W		41.571	51.401	87.864	1.00 31.72
ATOM	66	0	нон м		35.633	56.807		1.00 42.27
ATOM	67	0	HOH W		35.257	40.157	78.063	1.00 30.17
ATOM	68	O	HOH W		33.734	71.189	79.910	1.00 32.64
ATOM	69	0	HOH W		17.659	69.593	75.158	1.00 46.73
MOTA	70	0	нон м		17.005	72.932	72.774	1.00 33.93
ATOM	71	O	нон м		15.769	48.059	85.107	1.00 24.21
ATOM	72	О	HOH W		15.023	64.697	75.333	1.00 39,99
ATOM	73	O	HOH W		13.546	67.305	74.469	1.00 38.11
ATOM	74	0	нон м		30.044	75.863	82.738	1.00 29.02
ATOM	75	0	нон м		5.253	66.383	98.323	1.00 61.09
ATOM	76	0	HOH W		25.914	72.829	89.073	1.00 48.08
ATOM	77	0	HOH W		38.474	67.620	76.050	1.00 32.88
ATOM	78	0	HOH W		34.101	41.534		1.00 39.54
ATOM	79	0	HOH W	310	29.974	37.419	76.650	1.00 39.99

(Drawing 43)

ATOM	80	0	HOH W	311	17,829	44.406	81.773		29.29
ATOM	81	0		312	17.766	66.478	75.705		34.51
ATOM	82	0	HOH W		35,983	70.225	78.152		38.85
ATOM	83	O	HOH W	314	18.063	58.644	75.592		33.24
ATOM	84	0	HOH W		17.740	45.824	75.692		31.29
ATOM	85	0	HOH W		21.442		101.498		30.06
ATOM	86	0	HOH W		30.660		105.501		46.34
ATOM	87	0	HOH W		28.143	47.582	99.410		71.00
ATOM	88	٥	HOH W		11.398	65.394	76.821		34.86
ATOM	89	0	нон м		31.737	45.760	98.744		38.11
ATOM	90	0	нон м		16.084	45.559	87.137		43.68
MOTA	91	0	HOH M		36.498	37.962	78.989		35.45
MOTA	92	0	нон м		41.868	42.172	76.980		56.04
MOTA	93	O		324	44.704	68.004	76.606		73.28
MOTA	94	0	HOH W	325	30.214		101.119		28.63
ATOM	95	0	HOH W	326	43.719	69.244	83.004		32.20
ATOM	96	0	нон м	327	7,992	54.768	93.490		36.05
MOTA	97	0	нон м	328	11.059	49.604	75.476		43.80
MOTA	98	0	нон м		17.730	37.202	79.516		44.41
MOTA	99	0	нон м	330	14.170	59.796	74.913	1.00	70.26
MOTA	100	0	HOH W	331	28.648	70.326	88.645		34.35
ATOM	101	0	HOH W	332	16.146	57.197	73.492	1.00	49.27
MOTA	102	0	нон и	333	11.086	52.502	82,116	1.00	39.47
MOTA	103	0	HOH W	334	15.950	60,744	73.392	1.00	63.16
MOTA	104	O	HOH W	335	23.809	74.443	89.142		63.73
ATOM	105	0	HOH W	336	43.077	70.945	86.543		41.77
MOTA	106	0	HOH W	337	44.625	68.578	85.466	1.00	42.53
ATOM	107	0	HOH W	338	38.003	70.941	79.707	1.00	47.97
MOTA	108	0	нон и	339	42.635	39.826	86.317		39.90
MOTA	109	0	HOH W	340	28.158	51.028	97.893	1.00	35.28
ATOM	110	0	HOH W		34.562	57.666	98.193	1.00	56.42
MOTA	111	0	HOH W	342	23.659	34.535	79.197		84.39
MOTA	112	O	HOH W	343	10.337	58.458	76.704		45.85
MOTA	113	0	W HOH	344	32.164	75.101	85.461	1.00	54.21
ATOM	114	0	HOH W	345	32.930	38,410	86.586		43.15
ATOM	115	0	нон м	346	32.310	36.987	102,558	1.00	47.71
MOTA	116	0	HOH W	347	11.163	49.101	82.634	1.00	84.37
ATOM	117	0	нон м	348	34.268	69.634	83.019	1.00	47.39
MOTA	118	0	HOH W	349	31,352	37.085	89.579	1.00	74.88
ATOM	119	0	нон w	350	29.118	56.986	95.860	1.00	34.59
ATOM	120	Q	нон w	351	1.634	70.786	81.659	1.00	41.89
MOTA	121	0	нон м	352	2.044	71.714	85.736	1.00	37.84
MOTA	122	0	нон м	353	16.219	75.511	74.471	1.00	44.53
ATOM	123	0	нон м	354	24.035	45.705	97.204	1.00	48.11
MOTA	124	0	нон м	355	17.939	77.382	82.853	1.00	65.65
MOTA	125	0	W HOH		12.504	76.991	70.634	1.00	50.43
MOTA	126	0	нон w	357	16.951	78.295	74.889	1.00	47.02
ATOM	127	0	нон м	358	15.777	75.404	81.566	1.00	33.68
MOTA	128	0	нон w		37.401	72.376	82.831	1.00	50.52
ATOM	129	0	нон w	360	14.060	44.359	88.918	1.00	80.84
ATOM	130	0	нон и	361	32.619	76.123	75.757	1.00	42.84
ATOM	131	0	HOH W	362	21.836	66.226	94.339	1.00	63.40
ATOM	132	0	нон w	363	16.011	46.526	82.837	1.00	38.42
ATOM	133	0	HOH W		7.716	57.886	82.470	1.00	50.22

(Drawing 44)

ATOM	1.34	0	HOH W		41.813	72.155	81.960		76.60
ATOM	135	0	HOH W		5.810	63.614	94.440		41.72
ATOM	136	0	HOH W		22,833	66.006	98.308		65.79
ATOM	137	0	нон w		21.384	36.791	76.692		59.29
ATOM	138	0	HOH W		38.765	52.950	92.219		27.87
ATOM	139	0	HOH W		46.430	68.991	81.609		70.52
ATOM	140	0	HOH W		36.973	69.709	83.153		33.81
ATOM	141	0	HOH W		22,238	43.716	92.825		44.49
MOTA	142	0	нон и		23.096	80.189	77.308		50.73
ATOM	143	0	нон и	374	2.790	53.932	81.478		50.22
MOTA	144	0	HOH W	375	3.292	64.768	94.055		46.64
ATOM	145	O	HOH W		26.937	79.257	75.755		47.21
ATOM	146	0	HOH W	377	45.046	50.594	85.873	1.00	46.10
ATOM	147	O	HOH W	378	24.988	68.312	90.158		39.03
ATOM	148	0	HOH W	379	2.045	61.203	93.643	1.00	49.73
MOTA	149	0		380	44.273	56.110	87,700	1.00	46.74
ATOM	150	O	нон w	381	26.747	76.462	73.043	1.00	50.91
ATOM	151	0	HOH W	382	40.545	70.889	76,918	1.00	68.80
ATOM	152	0	нон и	383	25.523	80.486	83.807	1.00	67.90
ATOM	153	0	нон w	384	40.972	36.296	87.372	1.00	66.49
ATOM	154	0	нон и	385	12.617	56.710	77.567	1.00	44.81
ATOM	155	0	нон и	386	44.460	48.054	74.082	1.00	41.02
ATOM	156	0	HOH W	387	35.781	73.896	86.117	1.00	38.14
ATOM	157	O	нон и	388	21.625	80.398	81.815	1.00	47.96
ATOM	158	0	HOH W	389	46.628	56.635	82.977	1.00	50.89
ATOM	159	0	нон и	390	12.308	51.573	78.083	1.00	64.92
ATOM	160	O	HOH W	391	30.773	39,420	87.798	1.00	55.92
ATOM	161	0	нон и	392	26.088	65.110	89.923	1.00	44.80
MOTA	162	0	HOH W	393	10.719	70.886	96.928	1.00	48.46
ATOM	163	Ø	нон w	394	12.474	47.243	84.457		53.08
ATOM	164	٥	нон и	395	24.296	71.312	91.828	1.00	48.39
ATOM	165	0	нон w	396	6.459	50.108	83.133	1.00	57.82
ATOM	166	0	нон и	397	42.423	66.213	75.196		34.97
ATOM	167	0	HOH W	398	29.045		101.769	1.00	46.59
ATOM	168	Ó	HOH W	399	27.195	39.655	105.406	1.00	41.59
ATOM	169	0	HOH W	400	6.834	56.385	96.211	1.00	45.81
ATOM	170	0	нон и	401	47.957	50.138	78.280	1.00	43.60
ATOM	171	0	нон и	402	23.330	36.461	72.787	1.00	54.41
ATOM	172	0	HOH W	403	29.051	79.533	81.900	1.00	78.87
ATOM	173	0	HOH W	404	46.670	55.026	74.340	1.00	68.61
ATOM	174	0	HOH W	405	28.985	78.746	85.840	1.00	75.16
MOTA	175	0	HOH W	406	32.117	68.589	73.365	1.00	42.10
ATOM	176	0	HOH W	407	48.677	52.842	75,727	1.00	66.77
ATOM	177	0	HOH W	408	29.185	36.245	72.017	1.00	
ATOM	178	0	HOH W	409	37.168	67.596	97.670	1.00	38.24
ATOM	179	0	HOH W	410	11.986	77,352	92.370		35.94
ATOM	180	0	HOH W		39.548	63.174	98.280		39.58
MOTA	181	0	HOH W		30.500	79.967	79.292		62.87
MOTA	182	0	нон w		18.003	41.205	83.764		64.48
ATOM	183	0	HOH W		34.455	37.242	89.080		52.58
MOTA	184	0	HOH W	415	47.074	60.938	83.746		66.98
ATOM	185	0	HOH W	416	10.880	54.535	78.559	1.00	45.29
ATOM	186	O	HOH W	417	30.230	76.830	74.341	1.00	71.09
ATOM	187	0	HOH W	41.8	12.118	81,147	79.341	1.00	49.66

[Drawing 45]

```
76.945
                                                   80.548
                                                           1.00 63.23
                  HOH W 419
                                  32.095
        188
             0
ATOM
                                                   84,539
                                                           1.00 48.83
        189
                  HOH W 420
                                  -0.301
                                          68.264
MOTA
                                  10.822
                                          64.227 102.313
                                                           1.00 81.32
        190
                  HOH W 421
ATOM
             O
                                          42.555 101.170
                                                           1.00 42.91
                  HOH W 422
                                  23.374
             0
ATOM
        191
        192
             0
                  HOH W 423
                                  20.016
                                          59.713
                                                   74.793
                                                           1.00 38.45
ATOM
                                                   79,495
                                                           1.00 56.01
                                  15.833
                                          78.412
ATOM
        193
             Q
                  HOH W 424
                                                   84.957
                                                           1.00 63.16
        194
                  HOH W 425
                                  43.534
                                          35.850
MOTA
             0
                                  11.933
                                          68.018
                                                   98,874
                                                           1.00 52.15
ATOM
        195
             0
                  HOH W 426
                                  20.777
                                          37.368
                                                   85.962
                                                           1.00 57.71
MOTA
        196
             0
                  HOH W 427
                                                   89.560
                                                           1.00 68.43
                  HOH W 428
                                  22.392
                                          36.632
        197
ATOM
             O
                                  29.340
                                          37.487 101.980
                                                           1.00 74.20
        198
                  HOH W 429
             0
ATOM
                                                   91.878
                                                           1.00 74.07
ATOM
        199
             0
                  HOH W 430
                                  23.237
                                          39.294
                                                   94.697
                                                           1.00 73.83
                                  13.654
                                          75.325
MOTA
        200
             0
                  HOH W 431
                  HOH W 432
                                  27.904
                                          38.307
                                                   96.631
                                                           1.00 57.14
             0
ATOM
        201
                                          59.909
                                                   79.188
                                                           1.00 37.71
MOTA
        202
             0
                  HOH W 433
                                  44.213
                                                   79.755
                                   2,129
                                          75,408
                                                           1.00 64.17
        203
             Ö
                  HOH W 434
ATOM
                                  13.993
                                          43.469
                                                   84,483
                                                           1,00 59.78
        204
                  HOH W 435
MOTA
             0
                                                   99.951
                                                           1.00 58.81
                                  31.644
                                          55.529
MOTA
        205
             O
                  HOH W 436
                                   9.462
                                          82.415
                                                   76,470
                                                           1.00 48.44
                  HOH W 437
        206
             0
ATOM
                                  21,813
                                          58.761
                                                   98.061
                                                           1.00 60.37
        207
             0
                  HOH W 438
ATOM
                                                   93.382
                                                           1.00 43.39
ATOM
        208
             0
                  HOH W 439
                                  22.202
                                          59.533
                                                           1.00 46.86
                                                   86.455
                                  18.118
                                          43.497
        209
                  HOH W 440
MOTA
             0
                                  13.762
                                          54.340 105.466
                                                           1.00 57.78
                  HOH W 441
ATOM
        210
             0
                                                   83.853
                                                            1.00 56.73
                                  33.277
                                          73.931
        211
             0
                  HOH W 442
ATOM
                                                            1.00 27.90
        212
             0
                  HOH W 443
                                  34.442
                                          68.648
                                                   90.744
ATOM
                                                            1.00 53.48
                                          67.899
                                                   91.831
MOTA
        213
             0
                  HOH W 444
                                  30.640
                                                           1.00 53.35
                                                   74.058
                                          44.217
        214
             ٥
                  HOH W 445
                                  40.813
MOTA
                                          71.334
                                                  90.213
                                                           1.00 53.98
                                  33.012
                  HOH W 446
MOTA
        215
             0
                  HOH W 447
                                  25.130
                                          57.928 101.293
                                                           1.00 38.97
        216
             0
MOTA
                                   7.584
                                          82.067
                                                   74.163
                                                           1.00 26.55
        217
             0
                  HOH W 448
ATOM
                                                  78.980
                                                           1.00 37.75
        218
                  HOH W 449
                                  42.214
                                          40.521
ATOM
             0
                                   8.915
                                          57.776 101.115
                                                           1.00 50.37
                  HOH W 450
ATOM
        219
             0
                                                   79.699
                                                            1.00 71.24
                  HOH W 451
                                  15.963
                                          42.582
        220
             0
MOTA
                                  23.011
                                          77.967
                                                   75.363
                                                            1.00 64.04
        221
             0
                  HOH W 452
MOTA
        222
                  HOH W 453
                                  36.910
                                          35.452
                                                   88.469
                                                            1.00 70.47
MOTA
             0
                                                   99.966
                                                           1.00 54.36
                                          55.271
MOTA
        223
             Ø
                  HOH W 454
                                  37.814
                                                            1.00 86.46
                                                   99.230
                                          58.439
MOTA
        224
              0
                  HOH W 455
                                  26.721
                                          40.093
                                                   81.126
                                                            1.00 98.28
                  HOH W 456
                                  16.108
        225
MOTA
             0
                                          35.543
                                                   96.536
                                                            1.00 63.56
        226
             0
                  HOH W 457
                                  27.800
ATOM
                                   5.859
                                          51.318
                                                   95.801
                                                           1.00 68.96
        227
             0
                  HOH W 458
MOTA
                                           51.875
                                                   96.622
                                                            1.00 64,76
        228
                  HOH W 459
                                   7.841
ATOM
             0
                                                            1.00 73.27
        229
              Q
                  HOH W 460
                                  28.280
                                          66.535
                                                   89,122
MOTA
                                  13.943
                                           46.268
                                                   81.680
                                                            1.00 56.20
                  HOH W 461
ATOM
        230
             O
                                           69.220
                                                   73.344
                                                            1.00 83.46
        231
              ٥
                  HOH W 462
                                  14.681
ATOM
                                                   89.815
                                                            1.00 60.45
MOTA
        232
              0
                  HOH W 463
                                  30.388
                                          71.379
                                                   75.809
                                                            1.00 59.76
                                   8.062
                                           56.915
ATOM
        233
              0
                  HOH W 464
                                  30.104
                                           41,907 101.688
                                                           1.00 61.89
                  HOH W 465
MOTA
        234
             0
        235
             0
                  HOH W 466
                                   4,988
                                           49,407
                                                   95.471
                                                            1.00 61.48
ATOM
                                   8,747
                                          53.997 77.187
                                                           1.00 79.74
        236 O
                  HOH W 467
ATOM
END
```

```
Drawing 46]
          5'-CA-GAC-CTG-GCC-TTT-GGC-GAT-GTG-GC-3'
A72F(8)
          3'-GT-CTG-GAC-CGG-AAA-CCG-CTA-CAC-CG-5'
A72F(as)
                                           V
                 D
                      L
                              F72 G
                                       D
                          A
          5'-CA-GAC-CTG-GCC-GAA-GGC-GAT-GTG-GC-3'
A72E(s)
          3'-GT-CTG-GAC-CGG-CTT-CCG-CTA-CAC-CG-5'
A72E(as)
                 D
                      L
                          A
                              E72 G
                                       D
```

[Drawing 47]

```
I103D(s) 5'-TG-ACC-AAT-ATG-GAC-GAG-GAC-GCC-GG-3'
I103D(as) 3'-AC-TGG-TTA-TAC-CTG-CTC-CTG-CGG-CC-5'
                     N
                       M D103 E
T153N(s) 5'-GG-CAT-ACC-TCT-AAC-GGC-TGG-GCT-AC-3'
T153N(as) 3'-CC-GTA-TGG-AGA-TTG-CCG-ACC-CGA-TG-5'
                            N153 G
                 H
                     Т
                         S
                                         A
[Drawing 48]
L140F(s)
         5'-AC-CAG-GAC-AAA-TTC-TCC-AAA-AAT-GG-3'
L140F(as) 3'-TG-GTC-CTG-TTT-AAG-AGG-TTT-TTA-CC-5'
                     D K F140 S
                                     K
L140K(s) 5'-AC-CAG-GAC-AAA-AAA-TCC-AAA-AAT-GG-3'
L140K(as) 3'-TG-GTC-CTG-TTT-TTT-AGG-TTT-TTA-CC-5'
                 Q
                     D
                        K
                            K140 S
```

L140E(s) 5'-AC-CAG-GAC-AAA-GAA-TCC-AAA-AAT-GG-3'
L140E(as) 3'-TG-GTC-CTG-TTT-CTT-AGG-TTT-TTA-CC-5'

K

E140 S

ĸ

N

D

Q

[Translation done.]

* NOTICES *

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- 1. This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

WRITTEN AMENDMENT

______ [Written amendment]

[Filing date]September 14, Heisei 12 (2000.9.14)

[Amendment 1]

[Document to be Amended]Specification

[Item(s) to be Amended]0050

[Method of Amendment] Change

[Proposed Amendment]

[0050]Example 20 Enterobacter aerogenes IFO 12010 Refining of origin wild type acid phosphatase and determination of N-terminal-amino-acid arrangement

Refine acid phosphatase of the Enterobacter aerogenes IFO12010 origin from the culture object of Escherichia coli JM109/pENP110 of example 24 statement of JP,10-201481,A, and N-terminalamino-acid arrangement is determined, The amino acid sequence of maturation protein was determined. Escherichia coli JM109/pENP110 are the bacillus which introduced the acid phosphatase gene of the Enterobacter aerogenes IFO12010 origin into 109 shares of Escherichia coli JM, and produce this acid phosphatase. The amino acid sequence of the precursor protein expected from the base sequence of this acid phosphatase gene is equivalent to the arrangement shown in the array number 10 of an array table. The amino acid sequence shown in the array number 10 is an amino acid sequence of L61 Q/A63 Q/E64 A/N67 D/S69 A/G72 D/T133 K/E134 D/I151T variant EA-AP. 50 ml of nutrient media (pH 7.0) containing 1 g/dl of peptone, 0.5 g/dl of yeast extracts, and 1 g/dl of salt were put into a 500-ml Sakaguchi flask, and it heat-sterilized for 20 minutes at 120 **. One platinum loop of Escherichia coli JM109/pENP110 was inoculated into this, and shaking culture was carried out at 30 ** for 16 hours. The biomass which collected biomasses from culture medium by centrifugal separation was suspended to the 100mM potassium phosphate buffer (pH 7.0) of 100 ml, ultrasonication was performed for 20 minutes at 4 **, and the biomass was crushed. The treating solution was centrifuged and the cell-free extract was prepared except for the insoluble fraction. Ammonium sulfate was added so that it might become this cell-free extract with saturation 30%. After removing the precipitate generated by centrifugal separation, additional addition of the ammonium sulfate was carried out so that it might become digestive liquor with saturation 60%. Centrifugal separation recovered the generated precipitate and it dissolved in the 100mM potassium phosphate buffer. After dialyzing this crude enzyme liquid 3 times to 500 ml of 100mM potassium phosphate buffers (pH 7.0), It charged in DEAE-TOYOPARU 650M column (phi3.0x10.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0), and 20mM potassium phosphate buffer (pH 7.0) washed. Since phosphate transfer activity suited the bypassing fraction, the fractions concerned were collected. Ammonium sulfate was added so that it might become this activity fraction with saturation 35%, and this was made to stick to the butyl-Toyopearl column (phi3.0x7.0cm) equilibrated with 20mM potassium phosphate buffer (pH 7.0) which contains saturated ammonium sulfate 35%. This was eluted by the linear concentration gradient of the saturated potassium phosphate buffer (pH 7.0) 20% from saturation 35%. CM-TOYOPARU equilibrated with 10mM potassium phosphate buffer (pH 6.0) after collecting activity fractions and dialyzing to 10mM potassium phosphate buffer (pH 6.0) 1L It was made to stick to a column (phi3.0x7.0cm). This was eluted by the linear concentration gradient of the

potassium phosphate buffer (pH 6.0) containing 300mM potassium chloride from 0mM. These activity fractions were collected. The above operation refined the enzyme in which phosphate transfer activity is shown about 5 times with the recovery rate of about 16% more nearly eventually than a cell—free extract. This enzyme preparation was uniform in SDS—polyacrylamide electrophoresis. It is DITC about these refining enzymes. Membrane It is made to stick to [a milli gene / bio—search (Milligen/Biosearch) company make], When the amino acid sequence of the amino terminal was determined using Prosequencer 6625 (a milli gene / bio—search company make), the amino acid sequence of the amino terminal of 5 residue shown in the array number 98 of the array table was determined. Since the amino terminal of refining enzymes was started from the 21st alanine residue of the arrangement of the array number 10 of an array table, The amino acid sequence shown in the array number 10 of an array table is the arrangement of a precursor protein, and it was thought that peptide to the 20th phenylalanine residue was removed from the 1st methionine residue after translation. From this result, the amino acid sequence of mature—bodies protein is equivalent to the arrangement shown in the amino acid numbers 1–228 during the arrangement shown in the array number 10 of an array table.

[Amendment 2]

[Document to be Amended] Specification

[Item(s) to be Amended]0062

[Method of Amendment] Change

[Proposed Amendment]

[0062]Example 23 Enterobacter aerogenes whose compatibility over guanosine improved IFO 12010 Phosphorylation of the guanosine by an origin new variant acid phosphatase transgenics bacillus Each variant acid phosphatase gene. The included plasmid. introduced Escherichia coli .

JM109/pENP180, Escherichia coli JM109/pENP320, Escherichia coli JM109/pENP340, Escherichia coli JM109/pENP410, Escherichia coli JM109/pENP510, And Escherichia coli JM109/pENP520 was inoculated into 50 ml of L culture media containing ampicillin 100microg/ml and IPTG1mM, and it cultivated at 37 ** for 16 hours. 10 g/dl of pyrophoric acid and 6.6 g/dl of grinding guanosine prepared like Example 1 of the Japanese-Patent-Application-No. No. 189226 [12 to] specification are dissolved in a 100mM acetic acid buffer (pH 4.5), It was made to react at 35 ** for 12 hours, maintaining [to this, add each biomass so that it may become 100 mg/dl by dry cell weight, and] pH to 4.5. The quantity of generated 5'-guanylic acid was shown in Table 17. Escherichia coli each whose bacillus which introduced the enzyme variant as shown in Table 17 is an old stock Productivity improved compared with JM109/pENP180, and generation accumulation of the 5'-guanylic acid was carried out with high yield.

[Translation done.]

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(54) 【発明の名称】 変異型ヌクレオシド-5'ーリン酸生産酵素

(57) 【要約】 (修正有)

【課題】 ヌクレオシドー5′ーリン酸生産能が向上した新規な変異型ヌクレオシドー5′ーリン酸生産酵素、その取得のための新規な手段、及び該酵素の用途を提供する。

【解決手段】 ヌクレオシドー5′ーリン酸生産酵素において、Lys残基1つ、Arg残基2つ、His残基2つが存在し、これらのCα間距離が特定の範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び/又はホスファターゼ活性を有する酵素のヌクレオシドー5′ーリン酸生産能が向上した変異型ヌクレオシドー5′ーリン酸生産酵素。既知の酵素の結晶のX線構造解析に基づく変異の確定による該酵素の製造方法。

【効果】 より活性の高い酵素の取得が容易となった。

【特許請求の範囲】

【請求項1】 ヌクレオシド-5′ーリン酸生産酵素において、Lys残基1つ、Arg残基2つ、His残基2つが存在し、これらのCα間距離が図1に示す範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び/又はホスファターゼ活性を有する酵素のヌクレオシド-5′ーリン酸生産能が向上した変異型ヌクレオシド-5′ーリン酸生産酵素。

【請求項2】 酵素の由来がエシェリヒア属細菌、モルガネラ属細菌、プロビデンシア属細菌、エンテロバクター属細菌、クレブジエラ属細菌、又はエンテロバクター属細菌に属するものである請求項1に記載の変異型ヌクレオシド-5′ーリン酸生産酵素。

【請求項3】 エシェリヒア・ブラッタエ由来酸性ホスファターゼの結晶を X 線結晶構造解析して得られる原子座標データに示されている構造座標を元にして、イノシン、グアノシン等のヌクレオシド並びにそのリン酸化合物との結合様式を推定し、アミノ酸残基及び/又は補欠因子等の置換、追加、削除によりヌクレオシドー5′ーリン酸生産能が向上した請求項1記載の変異型ヌクレオシドー5′ーリン酸生産酵素。

【請求項4】 エシェリヒア・ブラッタエ由来酸性ホスファターゼのアミノ酸配列の以下の位置(エシェリヒア・ブラッタエ酸性ホスファターゼのSer 7 2、又はSer 7 2から10Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、140、148-154、157、179、183の少なくとも一つの位置に変化が生じている変異型ヌクレオシドー5′ーリン酸生産酵素。

【請求項5】 リン酸基転移活性及び/又はホスファターゼ活性を有する酵素で、エシェリヒア・ブラッタエ由来酸性ホスファターゼとのアミノ酸配列アラインメントをしたときに、エシェリヒア・ブラッタエ由来酸性ホスファターゼのの世間(エシェリヒア・ブラッタエ酸性ホスファターゼのSer 7 2、又はSer 7 2から10Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、140、148-154、157、179、183に対応する少なくとも一つの位置に変化が生じている変異型ヌクレオシド-5′ーリン酸生産酵素。

【請求項6】 リン酸基転移活性及び/又はホスファターゼ活性を有する酵素で、エシェリヒア・ブラッタエ由来酸性ホスファターゼの3次元構造とのアラインメントをトレッディング法により行ったときに、エシェリヒア・ブラッタエ由来酸性ホスファターゼのアミノ酸配列の以下の位置(エシェリヒア・ブラッタエ酸性ホスファターゼのSer 7 2、又はSer 7 2から10 Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、14

0、148-154、157、179、183に対応する少なくとも一つの位置に変化が生じている変異型ヌクレオシド-5'-リン酸生産酵素。

【請求項7】 酵素の由来がエンテロバクター・アェロゲネス由来であり、そのアミノ酸配列において14番目のロイシン残基、61番目のロイシン残基、63番目のアラニン残基、64番目のグルタミン酸残基、70番目のアスパラギン残基、69番目のセリン残基、70番目のアラニン残基、71番目のグリシン残基、72番目のグリシン残基、102番目のグルタミン酸残基、133番目のスレオニン残基、134番目のグルタミン酸残基、138番目のロイシン残基、149番目のスレオニン残基、151番目のイソロイシン残基、149番目のスレオニン残基、151番目のイソロイシン残基のうち少なくとも一つのアミノ酸残基が他のアミノ酸残基に置換されたものである変異型ヌクレオシド-5′ーリン酸生産酵素。

【請求項8】 リン酸基転移活性及び/又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体の結晶をX線結晶構造解析して得られる立体構造から確定された、該酵素の活性部位、及び/又はそれから10Å以内にあるアミノ酸残基を、置換、追加、削除をすることにより、ヌクレオシド-5′ーリン酸生産能が向上した変異型酵素を製造することを特徴とする変異型ヌクレオシド-5′ーリン酸生産酵素の製造方法。

【請求項9】 エシェリヒア・ブラッタエ由来酸性ホスファターゼの構造座標を使用して、ホスファターゼ又はリン酸基転移酵素の阻害剤を製造する方法。

【請求項10】 リン酸基転移活性及び/又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体のいずれかの結晶。

【請求項11】 六方晶系の空間群P6322を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼの結晶。

【請求項12】 斜方晶系の空間群P2₁2₁2₁を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼG74D/I153T変異型酵素の結晶。

【請求項13】 三方晶系の空間群P3₁21を有する、エシェリヒア・ブラッタエ由来酸性ホスファターゼとモリブデン酸との複合体(反応中間体アナログ)の結晶。

【請求項14】 請求項1~7のいずれか1項に記載の 酵素をコードする遺伝子。

【請求項15】 請求項14に記載の遺伝子を含む組換 えDNA。

【請求項16】 請求項15に記載の組換えDNAを保有する微生物。

【請求項17】 請求項1~7のいずれか1項に記載の 酵素、又はそれを含有する微生物、あるいは請求項16 に記載の微生物を、ヌクレオシド並びにリン酸供与体に 作用させてヌクレオシドー5′ーリン酸を生成させ、こ れを採取することを特徴とするヌクレオシドー 5′-リン酸の製造方法。

【発明の詳細な説明】

[0001]

【発明の属する技術分野】本発明は、ヌクレオシドー 5'ーリン酸生産能が向上した変異型ヌクレオシドー 5'ーリン酸生産酵素、及びその製造方法に関する。また、本発明は、上記した酵素の製造に有用な酵素類に関する。更に本発明は、ヌクレオシドー5'ーリン酸の製造方法に関し、またその製造方法に有用な、前記の変異型酵素をコードする遺伝子、該遺伝子を含む組換えDNA、該組換えDNAを保有する微生物に関する。ヌクレオシドー5'ーリン酸は、調味料、医薬並びにそれらの原料として有用である。なお、本発明は、X線結晶構造解析技術により蛋白質の新規立体構造の解明に成功したことに基づくと共に、該立体構造は微生物に限らない発展性を有する。

【従来の技術】ヌクレオシドを生化学的にリン酸化して

[0002]

ヌクレオシドー5′ーリン酸を安価かつ効率的に製造す る方法として、特定の微生物菌体を、酸性条件下でヌク レオシド並びにポリリン酸(塩)、フェニルリン酸 (塩) 及びカルバミルリン酸から成る群より選択される リン酸供与体に作用させることにより、ヌクレオシドー 2′ーリン酸、ヌクレオシドー3′ーリン酸異性体の副 生を伴うことなくヌクレオシドー5′ーリン酸を効率よ く生成する方法が開発されている(特開平7-2317 93号)。その後、エシェリヒア・ブラッタエ(Escher ichia blattae) 及びモルガネラ・モルガニ(Morganell a morganii) より酸性ホスファターゼをコードする遺伝 子を取得し、遺伝子工学的に該遺伝子をエシェリヒア・ コリで大量発現することによりヌクレオシドー5′ーリ ン酸の生産性が更に向上することが確認された。該酸性 ホスファターゼの構造を図2に示す。すなわち、図2 は、エシェリヒア・ブラッタエ由来酸性ホスファターゼ (以下、EB-APと略記する)のアミノ酸配列を、モ ルガネラ・モルガニ, サルモネラ・チフィムリウム (Sa Imonella typhimurium), ザイモモナス・モビリス (Zy momonas mobilis) 由来酸性ホスファターゼのアミノ酸 配列とアラインメントした図である。それぞれの酸性ホ スファターゼの遺伝子の塩基配列と、コードされる酵素 のアミノ酸配列を、配列表の配列番号1~8に示す。図 2で星印は保存された残基を示す。2次構造の領域をア ラインメントの上に棒で示した。四角の線で囲んだ部分 は、酸性ホスファターゼファミリーの間で共通している モチーフを示す。モチーフは、1) KXXXXXRP配列番号 121、2) PSGH配列番号122、3) SRXXXXXHXXXD配 列番号123、の3つのドメインから成り立っている。 ここで、Xは任意のアミノ酸である。該酸性ホスファタ ーゼ(図2)は、リン酸基転移活性を有するものの、野 生型においては、ヌクレオシドー5′ーリン酸をヌクレ オシドに分解するホスファターゼ活性が優勢であり、蓄 積されたヌクレオシドー5′ーリン酸が分解されてしま う欠点があった。そこで、ランダムに多数の変異型酵素 を発生させ、その中からホスファターゼ活性に比してリ ン酸基転移活性が相対的に向上した変異型酸性ホスファ ターゼが見出され、該変異型酸性ホスファターゼ遺伝子 を大量発現させることによりヌクレオシドー5′ーリン 酸の生産性が飛躍的に向上することが示された(特開平 9-37785号)。該変異型ホスファターゼは、ヌク レオシドに対する親和性が向上しており、それによりリ ン酸基転移活性が改善されたと考えられる。上記のエシ ェリヒア・ブラッタエ由来変異型酸性ホスファターゼ (=G74D/I153T変異型酵素) は、モルガネラ ・モルガニ由来酸性ホスファターゼ(MM-AP)の対 応するG72D/I151T変異型酵素よりリン酸基転 移活性が弱いが、8つのアミノ酸残基を一次構造上対応 するMM-APのアミノ酸に置換した10残基置換L6 3Q/A65Q/E66A/N69D/S71A/S7 2A/G74D/T135K/E136D/I153T 変異型酵素(以降、単に10残基置換変異型EB-AP と記述) は、G72D/I151T変異型MM-APと ほぼ同等のリン酸基転移活性を得ることが示された(特 開平10-201481号)。

[0003]

【発明が解決しようとする課題】上述の生産性が向上したエシェリヒア・ブラッタエ由来酸性ホスファターゼ(EB-AP)のG74D/I153T変異型酵素遺伝子、更には10残基置換変異型酵素遺伝子をエシェリヒア・コリで大量発現することによりヌクレオシドー5′ーリン酸を生産する方法が確立された(特開平9-37785号、特開平10-201481号)が、更に生産性の向上した変異型EB-APが望まれる。本発明は、EB-APの3次元構造に基づいて、変異型EB-APを設計することにより、ヌクレオシド-5′ーリン酸生産性の更なる向上を図ることを課題とする。

[0004]

【課題を解決するための手段】本発明を概説すれば、下記に列挙するとおりである。

- (1) ヌクレオシドー5'ーリン酸生産酵素において、Lys残基1つ、Arg残基2つ、His残基2つが存在し、これらのCα間距離が図1に示す範囲内にあり、かつその付近にヌクレオシドが結合するスペースを有する、リン酸基転移活性及び/又はホスファターゼ活性を有する酵素のヌクレオシドー5'ーリン酸生産酵素。
- (2) 酵素の由来がエシェリヒア属細菌、モルガネラ 属細菌、プロビデンシア属細菌、エンテロバクター属細 菌、クレブジエラ属細菌、又はエンテロバクター属細菌 に属するものである(1)項記載の変異型ヌクレオシド

-5′ーリン酸生産酵素。

- (3) EB-APの結晶をX線結晶構造解析して得られる原子座標データに示されている構造座標を元にして、イノシン、グアノシン等のヌクレオシドとの結合様式を推定し、アミノ酸残基及び/又は補欠因子等の置換、追加、削除をすることによりヌクレオシドー5′ーリン酸生産能が向上した(1)項記載の変異型ヌクレオシドー5′ーリン酸生産酵素。
- (4) EB-APのアミノ酸配列の以下の位置(EB-APのアミノ酸Ser72、又はSer72から10Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、140、148-154、157、179、183の少なくとも一つの位置に変化が生じている変異型ヌクレオシド-5′-リン酸生産酵素。
- (5) リン酸基転移活性及び/又はホスファターゼ活性を有する酵素で、EB-APとのアミノ酸配列アラインメントをしたときに、EB-APのアミノ酸配列のの以下の位置(EB-APのアミノ酸Ser72、又はSer72から10Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、140、148-154、157、179、183の少なくとも一つの位置に変化が生じている変異型ヌクレオシド-5′ーリン酸生産酵素。
- (6) リン酸基転移活性及び/又はホスファターゼ活性を有する酵素で、EB-APの3次元構造とのアラインメントをトレッディング法により行ったときに、EB-APのアミノ酸配列のの以下の位置(EB-APのアミノ酸Ser72、又はSer72から10Å以内にある残基):16、67-76、78-79、96、99-100、102-104、106-108、115、140、148-154、157、179、183の少なくとも一つの位置に変化が生じている変異型ヌクレオシド-5'-リン酸生産酵素。

【0005】(7) 酵素の由来がエンテロバクター・アェロゲネス由来であり、そのアミノ酸配列において14番目のロイシン残基、61番目のロイシン残基、63番目のアラニン残基、64番目のグルタミン酸残基、67番目のアスパラギン残基、69番目のセリン残基、70番目のアラニン残基、71番目のグリシン残基、72番目のグリシン残基、101番目のイソロイシン残基、102番目のグルタミン酸残基、133番目のスレオニン残基、134番目のグルタミン酸残基、138番目のロイシン残基、149番目のスレオニン残基、151番目のイソロイシン残基のうち少なくとも一つのアミノ酸残基が他のアミノ酸残基に置換されたものである変異型ヌクレオシド-5′ーリン酸生産酵素。

(8) リン酸基転移活性及び/又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体の結晶をX線結晶構造解析して得られる立体構造から

確定された、該酵素の活性部位に位置するアミノ酸残基(Lys1つ、Arg2つ、His2つにより構成される)、及び/又はそれから10Å以内にあるアミノ酸残基を、置換、追加、削除をすることにより、ヌクレオシドー5′ーリン酸生産能が向上した変異型酵素を製造することを特徴とする変異型ヌクレオシドー5′ーリン酸生産酵素の製造方法。

(9) エシェリヒア・ブラッタエ由来酸性ホスファターゼの構造座標を使用して、ホスファターゼ又はリン酸 基転移酵素の阻害剤を製造する方法。

【0006】(10) リン酸基転移活性及び/又はホスファターゼ活性を有する酵素、あるいはそれとモリブデン酸との複合体のいずれかの結晶。

- (11) 六方晶系の空間群P6322を有する、EB-APの結晶。
- (12) 斜方晶系の空間群P2₁2₁2₁を有する、EB-AP G74D/I153T変異型酵素の結晶。
- (13) 三方晶系の空間群P3₁21を有する、EB-APとモリブデン酸との複合体(反応中間体アナログ)の結晶。

【0007】 (14) (1) ~ (7) 項のいずれか1 項に記載の酵素をコードする遺伝子。

- (15) (14) 項に記載の遺伝子を含む組換えDNA。
- (16) (15)項に記載の組換えDNAを保有する 微生物。

【0008】(17) (1)~(7)項のいずれか1項に記載の酵素、又はそれを含有する微生物、あるいは(16)項に記載の微生物を、ヌクレオシド並びにリン酸供与体に作用させてヌクレオシドー5′ーリン酸を生成させ、これを採取することを特徴とするヌクレオシドー5′ーリン酸の製造方法。

【0009】本発明は、EB-APの3次元構造を基に、ヌクレオシドとの結合様式モデルを構築し、それに基づき設計した変異型EB-APを利用したヌクレオシド-5′-リン酸の生産方法を提供する。

[0010]

【発明の実施の形態】以下、本発明を具体的に説明する。

(1) X線結晶構造解析により蛋白質の3次元構造を決定するには、蛋白質を結晶化する必要がある(実施例1-3に詳細を示した)。蛋白質を結晶化するためには、pH、バッファーの種類、バッファーの濃度、沈殿剤の種類、沈殿剤の濃度、金属等の添加剤の濃度、蛋白質の濃度、蛋白質の純度、等、数多くのパラメーターを試行錯誤により決定しなくてはならない。したがって、結晶を得るまでに数ヶ月~数年の時間がかかるのが通常であり、多大な労力に反して結晶が得られないケースも多々ある。結晶化は、3次元構造決定のためには欠かせないが、それ以外にも、蛋白質の高純度の精製法、高密

度でプロテアーゼ抵抗性の強い安定な保存法、更には酵素の固定化利用に先立つプロセスとして産業上の有用性 もある。

- (2) 作製した結晶に X線を照射して回折データを収集する。蛋白質結晶は X線照射によりダメージを受け回折能が劣化するケースが多々ある。その場合、結晶を急激に-173℃程度に冷却し、その状態で回折データを収集する低温測定が最近普及しつつある。なお、冷却に際しては、結晶が崩壊せず系全体がガラス状になるよう溶媒組成を工夫する必要がある。
- 結晶構造解析を行うには、回折データに加え て、位相情報が必要になる。EB-APは、類縁の蛋白 質の立体構造が未知であるため、重原子同型置換法によ り位相問題が解決されなくてはならない。重原子同型置 換法は、水銀や白金等原子番号が大きな金属原子を結晶 に導入し、金属原子の大きなX線散乱能のX線回折デー タへの寄与を利用して位相情報を得る方法である。野生 型EB-APの立体構造が決定されれば、変異型酵素及 び反応中間体アナログ等類縁体の結晶構造は、それを用 いた分子置換法により決定できる。分子置換法は、結晶 構造を決定したい蛋白質に類縁の蛋白質の立体構造が既 知の場合、その立体構造を利用して構造決定を行う手法 である。例えば、ある蛋白質の野生型の立体構造が分か っていれば、その変異型蛋白質や化学修飾された蛋白質 の結晶構造決定には、分子置換法が適用できる。G74 D/I153T変異型EB-APについては、2ヶ所の アミノ酸置換によるヌクレオシド親和性向上の分子機構 解明のために結晶構造を決定する。反応中間体アナログ については、ヌクレオシドとの結合様式モデルを構築す るために結晶構造を決定する。ヌクレオシドは、供与さ れるリン酸基が共有結合した状態のEB-AP、すなわ ち反応中間体に結合した後、ヌクレオシドー5′ーリン 酸に変換される。EB-APの反応中間体は不安定なの で、その構造を捉えることができないが、リン酸の代り にモリブデン酸が共有結合した反応中間体アナログであ れば加水分解されることがないので構造決定可能と考え た。実施例4、6、7に詳細を示した。
- 【0011】(4) コンピューターグラフィックス (CG) 上で、反応中間体アナログの3次元構造におけるモリブデン酸結合位置を基に、その付近の窪みにヌクレオシドをフィットさせ、結合様式モデルを構築する (図3)。モデルの構築には、例えばMSI社 (アメリカ)のQUANTA, INSIGHT IIの様なプログラムを利用する。なお、図3は、上記の結合様式モデルの結晶構造を示す写真である。実施例5、8に詳細を示した。
- (5) 結合モデルを良く観察し、ヌクレオシドとの親和性を増大させる変異を設計する。親和性を向上させるには、疎水相互作用・静電相互作用・水素結合・π-π相互作用(芳香環の環電流が発生する磁場同士の相互作用)・CH/π相互作用(芳香環の環電流とメチル基の

電子が発生する磁場の相互作用)を増強する手段が考え られる。Ser 7 2 はヌクレオシドの塩基と最も強く相互 作用すると予測されるため、Phe、Tyr、Trpへの置換は 疎水性相互作用及びπーπ相互作用を、Va1、IIe、Le uへの置換は疎水性相互作用及びCH/π相互作用をGlu、 Aspへの置換は静電相互作用及び水素結合を増強するも のと思われる。また、他のアミノ酸への置換、特により 長鎖の側鎖を有するアミノ酸への置換によっても疎水性 相互作用等が増強される可能性がある。Leu 16、Ser 7 1、Ser 7 3、Glu 1 0 4をPhe、Tyr、Trpに置換するこ とによっても置換されたアミノ酸残基の芳香環とヌクレ オシドの塩基との間にπーπ相互作用が形成されること が期待される。またIle103やThr153をより長鎖の 親水性残基に置換することによりヌクレオシドのリボー スとの水素結合の形成が期待される。更に、ヌクレオシ ド結合部位の近くに位置し、蛋白質内部に埋もれている Thr 151をSer、Ala、Glyといった小さな側鎖を持った アミノ酸残基に置換すれば、蛋白質内部に空隙が生じる ために、ヌクレオシド結合部位の柔軟性が増し、ヌクレ オシドとの結合により適したコンフォメーションを取れ るのではないかと期待される。なお、Leu 1 4 0 は、Ser 72から10 Å以上離れているが、反応中間体アナログ の立体構造において、リン酸結合部位の直近に位置す る。したがって、この残基を置換すれば、反応中間体に おけるリン酸結合部位周辺の構造が変化し、ひいては、 ヌクレオシド結合部位の構造と揺らぎにも影響が及ぶも のと考えられた。この残基を、よりかさ高いPhe、正電 荷を有するLys、負電荷を有するGlu、に置換すれば、ヌ クレオシドとの親和性が変化するのではないかと期待さ れる。上記の変異は、当初はG74D/I153T変異 型EB-APに対して導入する。しかし、導入の対象と する変異型酵素は、G74D/I153T変異型EB-APに限られるものではない。例えば、10残基置換変 異型EB-APに変異を導入することも可能である。変 異は、G74D/I153T変異型EB-APに対して 導入することにする。この場合、作製される変異型酵素 は、3残基置換変異型酵素となる。実施例9に詳細を示 した。

(6) PCR法により、変異型EB-APをコードする遺伝子を含むプラスミドを作製する。プラスミドをエシェリヒア・コリ(Escherichia coli)JM109に導入し、変異型EB-APを生産させる。変異型EB-APのイノシンに対する親和性の指標になるKm値、及びイノシンを5′ーイノシン酸に変換するリン酸基転移活性を測定し、変異型EB-APの性能を評価する。ヌクレオシド-5′ーリン酸の生産量は、多分にKm値に依存すると考えられる。ピロリン酸がEB-APと反応し、リン酸イオンが離脱し、リン酸基がEB-APと共有結合した形の反応中間体が形成された後、水分子がこれを攻撃すれば、リン酸基が外れてしまう(ホスファタ

ーゼ反応)。ピロリン酸は、ヌクレオシドー5′ーリン 酸を生成せずに無駄に消費されたことになる。一方、ヌ クレオシドが反応中間体を攻撃すれば、リン酸基はヌク レオシドとホスホモノエステル結合を形成し、生成した ヌクレオシドー5′ーリン酸がEB-APから離脱する (リン酸基転移反応)。ピロリン酸はヌクレオシドー 5′ーリン酸生成に活用されたことになる。つまり、反 応中間体を水とヌクレオシドが取り合い水が勝てばホス ファターゼ活性が、ヌクレオシドが勝てばリン酸基転移 活性が、発揮されることになる。ヌクレオシドのEB-APに対する親和性が上昇すれば、すなわち、Km値が 低下すれば、リン酸基転移反応が行われる可能性が高く なる。また、リン酸結合部位付近の疎水性を高め、水が 近づきにくくなれば、ホスファターゼ活性が弱まり、リ ン酸基転移活性が相対的に強まることになる。実施例1 0に詳細を示した。

- (7) Km値が低下し、かつリン酸基転移活性が上昇した変異型EB-AP遺伝子を含むプラスミドを導入したエシェリヒア・コリJM109を用いて、イノシンから5'ーイノシン酸を生産する実験を行う。30℃で45時間反応を行い5'ーイノシン酸生産量の経時変化をモニターする。実施例12に詳細を示した。
- (8) Kmを低下させる変異箇所が見出されたら、複 数の残基を組合せることによって更にヌクレオシドに対 する親和性が向上し、生産性の高くなった変異型酵素を 作製することができる。累加的に部位特異的変異を繰り 返すことで複数の変異部位を導入することができる。ま た部位特異的変異の導入の際に、変異を導入するアミノ 酸残基をコードする塩基の部分がミックス塩基となった プライマーを用いると特定のアミノ酸残基がすべてのア ミノ酸に置換された変異型遺伝子のライブラリーを作成 することができる。複数の部位にミックス塩基のプライ マーを用いて変異を導入すると非常に多種類の変異型酵 素をコードする変異型遺伝子のライブラリーを作成する ことができる。このようにして構築した遺伝子のライブ ラリーをエシェリヒア・コリに導入し、発現させたライ ブラリーから高活性のアミノ酸置換が組合された変異体 を選び出す方法も有効である。

【0012】EB-AP以外でも、EB-APと同様の活性部位及びヌクレオシドが結合しうるスペースを持つ

酵素であれば、ヌクレオシドー5′ーリン酸の生産に利 用できるポテンシャルがある。活性部位は、活性に必須 なアミノ酸残基を有し、かつ、それらが適切な空間的位 置関係で配置されていなくてはならない。EB-APに おいては、Lys115, Arg122, His150, Arg18 3, His189が活性に必須であり、これら5残基のC α間距離によって空間的位置関係を規定することが可能 である。本発明においては、野生型、G74D/I15 3 T変異型、反応中間体アナログの3つのEB-AP結 晶構造を決定したので、それぞれの構造における活性残 基の C α 原子間距離を測り、表 1 を作成した。表 1 の各 々の距離分布が約1 Å の幅を持つことから、最短距離よ り1 Å短い距離(表1、下限)以上、最長距離より1 Å 長い距離(表1、上限)以下であれば、求められる活性 部位を形成できるものと考えた。図1に、5つの残基の 位置関係を上限、下限の(α間距離と共に示した。な お、EB-APの類縁酵素であるMM-APにおいて、 活性残基間のすべての原子間距離が、EB-APの立体 構造から規定した範囲におさまっていることが確認され たことを実施例15に示した。本実施例では、野生型で はなく、G72D/I151T変異型の例を示したが、 同じ酵素の野生型と変異型で、活性部位の立体構造に大 きな違いはないと考えられる。この推測は、EB-AP の野生型とG74D/I153T変異型の活性部位の構 造が基本的に同じであることにより支持される(表1参 照)。ヌクレオシドをリン酸化して、ヌクレオシドー 5′ーリン酸に変換するには、上記の5残基から成る活 性部位だけでは不充分で、ヌクレオシドが適切な位置に 結合できなくてはならない。EB-APの場合は、リン 酸基の結合部位付近に、ヌクレオシドが結合するのに適 した溝状のスペースが分子表面に存在する〔(図3): 添付した原子座標を示す図10~図45を用いて、コン ピュータグラフィックス(CG)上で表示できる。〕こ の溝は、Leu 16、Ser 72、Glu 104、His 189の4 残基によって囲まれるスペースとして規定される。活性 部位を有していても、ヌクレオシドが結合する適切なス ペースがない酵素は、ヌクレオシドー5′ーリン酸生産 酵素としては不適である。

[0013]

【表1】

		野生型	G74D/I153T 变具型	反応中間体 アナログ	下限	上限
Lys 115	Arg122	11.6Å	11.6A	11.4Å	10.4A	12.6A
	Hist50	12.4Å	12. 3Å	12.8Å	11.3Å	13.8A
	Arg183	16.4A	16.3Å	15. 5Å	14.5A	17.4A
	His189	12.6A	12. 1 Å	11.7Å	10.7A	13.6Å
Argi 22	His150	13. 2Å	13. 6 A	14. 2Å	12. 2.A	15. 2Å
	Argl83	10.4Å	10.5Å	10.8A	9.4Å	11.8Å
	His189	5. 6Å	5. 5 A	5.7A	4.5A	6.7A
His150	Arg183	8.4A	8. 8 A	7.7Å	6.7A	9. 8A
	His189	9. 8Å	10.0A	10.0Å	8.8A	11.0A
Arg183	His189	5. 5 Å	5.8A	5.7Å	4.5Å	6.8Å

【0014】また、本発明は、Ser72の他のアミノ酸への置換、好ましくはPhe、Tyr、Trp、Val、Leu、Glu、Asp、Gln、Met、Thr、Arg、Lysのいずれか一つのアミノ酸への置換、を施した変異型EBーAPを提供する。更に、Ser72から10Å以内にある残基(残基番号:16、70-71,73-76、100、102-104、106-108、115、148-154、183)はヌクレオシドと相互作用する可能性が非常に高く、これらアミノ酸残基の他のアミノ酸への置換を施した変異型EBーAPを提供する。ここで置換とは、人為的にアミノ酸を置換した場合のみならず、自然界において置換が生じたEBーAPと同じ酵素ファミリーに属する他の酸性ホスファターゼを選抜することも包含する。ただし、本発明は、上記のアミノ酸残基以外の変異点を含む変異型EBーAPも提供できる。

【0015】また、EB-APと同じ酵素ファミリーに 属する他の酸性ホスファターゼにおいても、相同のアミ ノ酸変異を施せば、ヌクレオシドー 5′ーリン酸の製造 に利用することが可能である。ただし、EB-APのア ミノ酸残基が、他の酸性ホスファターゼにおいて同じ番 号のアミノ酸残基に対応するとは限らない。例えば、E B-APのSer72は、MM-APにおいてはAla70に 対応する。2つの異なった蛋白質のアミノ酸残基の対応 付けは、両者のアミノ酸配列の相同性が20%程度以上 であればアミノ酸配列同士のアラインメント(Sequence Alignment)、20%程度以下であれば3次元構造とア ミノ酸配列のアラインメント(Threading)により判明 する。前者はBLAST等、後者はINSIGHT II等のプログラ ムにより実行が可能である。BLASTを用いた、EB-A Pとエンテロバクター・アエロゲネス (Enterobacter a erogenes)由来酸性ホファターゼ(EA-AP)のアミ ノ酸配列アラインメントを実施例14に示した。BLAST は、FTPを使って、ncbi.nlm.nih.govより/blast/exec utableに存在するファイルのうち、使用するコンピュー ターに則したファイルを入手すればよい。操作法につい ては、http://genome. nhgri. nih. gov/blastall/blast#installに詳細が記述されている。

【0016】 ヌクレオシドー5′ーリン酸生産能向上 は、ヌクレオシドとの親和性向上により達成されるケー スが多いが、それ以外にも、至適pHのシフト、熱安定 性の向上、等によっても達成されうる。至適pHのシフ トは、活性残基のpKを変えることで達成できる〔プロ ティン エンジニアリング(Protein Engng.)、第1巻、 第383~388頁(1998)〕。熱安定性の向上は、プロリン残 基の導入、左巻きヘリックス構造を取る残基のグリシン 残基への置換〔プロティン エンジニアリング、第6 巻、第85~91頁(1993)〕、蛋白質内部の空隙を埋め ること〔バイオケミストリー(Biochemistry)、第32巻、 第6171~6178頁(1993)〕等により達成可能である。以上 詳細に説明したように、該立体構造はヌクレオシドの親 和性が向上し、ヌクレオシドー5′ーリン酸生産能の向 上した変異体を作製するために有効であるが、該立体構 造は酵素のヌクレオシドに対する親和性のみならず、リ ン酸供与体との親和性を変化させるのにも有効である。 特開平9-37785号公報に記載されているように該 酵素はポリリン酸(塩)、フェニルリン酸(塩)、アセ チルリン酸(塩)及びカルバミルリン酸(塩)等各種リ ン酸エステル化合物をリン酸供与体として利用すること が可能であるが、ヌクレオシドとの親和性を増大させる 変異を設計したのと同様の方法にて、リン酸エステル化 合物との親和性を増大させる変異を設計することで、リ ン酸供与体の基質特異性を広げたり、リン酸の利用率を 向上させることが可能である。

[0017]

【実施例】以下、本発明を実施例により更に具体的に説明するが、本発明はこれらの実施例に限定されない。

【0018】実施例1 野生型EB-APの結晶化 ハンギングドロップ法での蒸気拡散を利用して結晶化を 行った。野生型EB-AP(濃度10mg/ml)を含むリン酸ナトリウムの20mM緩衝液(pH8.0)と、 45(w/v%)のポリエチレングリコール400を含むトリス塩酸の100mM緩衝液を同量ずつ(各々7~10 μ I)、シリコナイゼイションしたカバーグラス上に滴下混合し、45(w/v%)のポリエチレングリコール400を含むトリス塩酸の100mM緩衝液500 μ Iを満たしたウエル(weII)の上に混合液滴が釣り下がるようにかぶせ、20%にて静置した。2、3日後に結晶が析出し、1週間から2週間後には測定可能な大きさ(0.3 \times 0.3 \times 1.2mm程度)の六角柱状の結晶に成長した。X線データ測定の際には、50(w/v%)のポリエチレングリコール400を含むトリス塩酸の100mM緩衝液(pH8.0)に結晶を移した。この結晶は、取扱い上、次の点に留意する必要があった。

1)液滴(ドロップレット)から、結晶を取り出す際に容器や用具に接触することで結晶が非常に崩れやすいため、シッティングドロップ法の結晶化形態は用いることが出来ず(結晶は成長するが)、ここで述べたハンギングドロップ法を用いた。2)常温測定では、測定中に結晶が劣化し、徐々に分解能が下がるため、低温条件下での測定が必要であった。結晶をステージにマウントするまでの時間を極力短くし、空気にさらさないように工夫した。(株)リガクのX線回折装置R-AXIS IIcを用いて、X線回折データを収集し、結晶学的パラメーターを決定した。空間群はP6322、格子定数は、a=b=124.4Å、c=97.7Åとなった。非対称単位に分子量25000のサブユニットを一つ含むと仮定すると、結晶の水分含有率は72%となる。

【0019】実施例2 G74D/I153T変異型EB-APの結晶化

ハンギングドロップ法での蒸気拡散を利用して結晶化を 行った。G74D/I153T変異型酵素(濃度20m g/ml) を含むトリス塩酸の20mM緩衝液(pH 8. 0)と、38 (w/v%) のポリエチレングリコー ル400を含むトリス塩酸の20mM緩衝液を同量ずつ (各々5 μ I)、シリコナイゼイションしたカバーグラ ス上に滴下混合し、38(w/v%)のポリエチレング リコール400を含むトリス塩酸の20mM緩衝液50 0 μ | を満たしたウエルの上に混合液滴が釣り下がるよ うにかぶせ、20℃にて静置した。2、3日後に結晶が 析出し、1週間後には測定可能な大きさ(0.7×0. 4×0. 2mm程度)の板状結晶に成長した。X線デー タ測定の際には、50(w/v%)のポリエチレングリ コール400を含むトリス塩酸の100mM緩衝液(p H8. 0) に結晶を移した。(株) リガクの X 線回折装置R -AXIS IIcを用いて、X線回折データを収集し、結晶学 的パラメーターを決定した。空間群はP212121、格 子定数はa=138.0Å、b=168.3Å、c=5 8. 2 Å となった。非対称単位に分子量 150000の 6量体分子を一つ含むと仮定すると、結晶の水分含有率 は64%となる。

【0020】実施例3 野生型EB-APとモリブデン 酸との複合体(反応中間体アナログ)の結晶化 シッティングドロップ法での蒸気拡散を利用した共結晶 化法を用いて結晶化を行った。野生型 EB-AP (濃度 10mg/ml) を含むリン酸ナトリウムの20mM緩 衝液(pH8.0)と、40(w/v%) のポリエチレン グリコール400、及び1mMのモリブデン酸ナトリウ ムを含むトリス塩酸の100mM緩衝液を同量ずつ(各 ϕ 15 μ I) を、40 (w/v%) のポリエチレングリ コール400を含むトリス塩酸の100mM緩衝液(p H8. 0)500 μ lを満たしたウェルに設置したブリ ッジの窪みに滴下混合し、20℃にて静置した。2~3 日後に結晶が析出し、1週間から2週間後には測定可能 な大きさ(0.3×0.3×0.3m程度)の菱餅状の 結晶に成長した。X線データ測定の際には、50(w/ v%) のポリエチレングリコール400を含むトリス塩 酸の100mM緩衝液(pH8.0)に結晶を移した。 (株)リガクのX線回折装置R-AXIS IIcを用いて、X線回 折データを収集し、結晶学的パラメーターを決定した。 空間群はP3121、格子定数はa=b=86.6Å、 c=205.3 Åとなった。非対称単位に分子量250 00のサブユニットを3つ含むと仮定すると、結晶の水 分含有率は58%となる。

【0021】実施例4 野生型EB-APの結晶構造解 析

最高1.9 Å分解能データまでのX線回折データを測定 した。結晶は、常温においては、X線の照射によるダメ ージが激しかったので、−173℃に急速冷却して測定 を行った。重金属塩類の溶液中に結晶を浸すことによ り、重原子誘導体のスクリーニングを行った。重原子誘 導体結晶の回折データはリガクR-AXIS IIcを用いて得 た。ネイティブデータとの差フーリエ図より、K₂P t C | 4が良好な重原子同型結晶を与えることを見出し た。プログラムRSPSを用いることにより、K2Pt C | 4の唯一のプラチナ結合部位の座標を求めた。この 座標をプログラムMLPHAREにより精密化し、それから計 算される位相を求めた。この位相を用い、2つ目の重原 子誘導体 K H g I 4 - K I の 5 カ所の水銀結合部位を求 めた。K2PtCl4、KHgl4-Kl両方の重原子パ ラメーターをMLPHAREを用いて同時に精密化した後、プ ログラムDMを用いて、溶媒平滑化とヒストグラムマッ チングを行い、位相を改良した。ちなみに、K2PtC 14については異常分散データも使用した。この良好な 位相を用いて計算した電子密度マップは非常に鮮明で、 ほとんどのアミノ酸残基をきれいにフィットすることが できた。最初のモデルは、2.8 Å分解能で作成した電 子密度マップ上でプログラムQUANTAを用いて構築し、プ ログラムX-PLORを用いて構造精密化を行った。N 末の6残基、135-136番目の残基、C末の1残基 は電子密度が観測されず、構造を一義的に決定できなか った。 1. 9 Å分解能で精密化された最終モデル(図4~図6)は、全231残基中222残基、236個の水分子、1分子の硫酸イオンを含む。硫酸イオンは、精製過程で用いた硫酸アンモニウムに由来しており、活性中心のリン酸結合部位に一致するものと考えられる。8~1. 9 Å分解能の反射を用いた結晶学的信頼度因子(R因子)は21. 5%となった。平均の温度因子は、蛋白質原子について26 Å2、水分子について45 Å2となった。プログラムPROCHECKを用いてラマチャンドランプロットを作成したところ、グリシン以外の残基の93%が最も好ましい領域に、7%が次に好ましい領域に位置することが示された。非対称単位にはサブユニット1個が含まれ、結晶学的対称性によって6量体が形成される。原子座標は図10~図45に示した。

【0022】なお、図4は、EB-APの6量体分子の 結晶構造を示すCG写真である。α炭素原子の流れをリ ボンモデルで表示した。また、活性中心をマークする硫 酸イオンをボールモデルで表示した。図5は、EB-A Pのサブユニットの結晶構造を示すCG写真である。 a 炭素原子の流れをリボンモデルで表示した。また、活性 中心をマークする硫酸イオンをボールモデルで表示し た。図6は、EB-APの活性部位構造を示す図であ る。中央に硫酸イオンを示した。また、水素結合を点線 で示した。図10は、EB-APの構造の結晶学データ (1)を示す図である。図11は、EB-APの構造の 結晶学データ(2)を示す図である。図12は、EB-APの構造の結晶学データ(3)を示す図である。図1 3は、EB-APの構造の結晶学データ(4)を示す図 である。図14は、EB-APの構造の結晶学データ (5) を示す図である。図15は、EB-APの構造の 結晶学データ(6)を示す図である。図16は、EB-APの構造の結晶学データ(7)を示す図である。図1 7は、EB-APの構造の結晶学データ(8)を示す図 である。図18は、EB-APの構造の結晶学データ (9) を示す図である。図19は、EB-APの構造の 結晶学データ(10)を示す図である。図20は、EB -APの構造の結晶学データ(11)を示す図である。 図21は、EB-APの構造の結晶学データ(12)を 示す図である。図22は、EB-APの構造の結晶学デ ータ(13)を示す図である。図23は、EB-APの 構造の結晶学データ(14)を示す図である。図24 は、EB-APの構造の結晶学データ(15)を示す図 である。図25は、EB-APの構造の結晶学データ (16)を示す図である。図26は、EB-APの構造 の結晶学データ(17)を示す図である。図27は、E B-APの構造の結晶学データ(18)を示す図であ る。図28は、EB-APの構造の結晶学データ(1 9) を示す図である。図29は、EB--APの構造の結 晶学データ(20)を示す図である。図30は、EB-APの構造の結晶学データ(21)を示す図である。図

31は、EB-APの構造の結晶学データ(22)を示 す図である。図32は、EB-APの構造の結晶学デー タ(23)を示す図である。図33は、EB-APの構 造の結晶学データ(24)を示す図である。図34は、 EB-APの構造の結晶学データ(25)を示す図であ る。図35は、EB-APの構造の結晶学データ(2 6)を示す図である。図36は、EB-APの構造の結 晶学データ(27)を示す図である。図37は、EB-APの構造の結晶学データ(28)を示す図である。図 38は、EB-APの構造の結晶学データ(29)を示 す図である。図39は、EB-APの構造の結晶学デー タ(30)を示す図である。図40は、EB-APの構 造の結晶学データ(31)を示す図である。図41は、 EB-APの構造の結晶学データ(32)を示す図であ る。図42は、EB-APの構造の結晶学データ(3 を示す図である。図43は、EB-APの構造の結 晶学データ(34)を示す図である。図44は、EB-APの構造の結晶学データ(35)を示す図である。図 45は、EB-APの構造の結晶学データ(36)を示 す図である。

【0023】実施例5 野生型EB-APと5'-イノシン酸の結合様式モデルの推測

イノシンのEB-APに対するKm値は100mMを超 えることから、結合様式をX線結晶構造解析で決定でき るほど親和性が高くない。実際に、グルコースー6ーサ ルフェートやアデノシンーチオモノホスフェートといっ たEB-APの阻害剤となる化合物を野生型EB-AP の結晶にソーキングした後、X線回折データを収集し、 電子密度マップを作成したが、これら化合物に対応する 電子密度は観測されなかった。そこで、コンピュータグ ラフィックスを用いて、5′ーイノシン酸とEB-AP の結合様式の推測(いわゆる、ドッキングスタディ)を 行うこととした。プログラムはQUANTAを用いた。結晶構 造中、活性部位中央に硫酸イオンが見出されたので、こ こに、5'ーイノシン酸のリン酸基を重ね合せた。更 に、G74D及びI153Tの変異が5'-イノシン酸 のEB-APに対するKm値を低下させることが知られ ていたので、5′ーイノシン酸はG74及び | 153か ら遠くないところに結合すると判断し、5′-イノシン 酸の位置を決めた。その際、5'-イノシン酸を構成す る原子とEB-APを構成する原子がお互いにぶつから ないようにした。こうして構築したモデルにおいて、I 153がTになると、置換されたスレオニンの側鎖のγ 酸素原子とイノシンのリボースの2′水酸基が水素結合 を形成する。また、プログラムGRASPを用いてEB-A Pの静電ポテンシャル表示をしたところ、正電荷を帯び るイノシン塩基は、EB-AP分子表面で負電荷を帯び ている領域と相互作用しており、モデルがもっともらし いことが示唆された。

【0024】実施例6 G74D/I153T変異型E

B-APの結晶構造解析

G74D/I153T変異型EB-APは、ホスファタ ーゼ活性に対するリン酸基転移活性の比率が高まってお り、それに伴い、ヌクレオシドー5'--リン酸の生産能 力も向上している。これは、ヌクレオシドとのKm値が 低下したこと、すなわち、ヌクレオシドとの親和性が向 上したことが原因として考えられている。この変異型E B-APの結晶構造を決定し、野生型EB-APの結晶 構造と比較すれば、ヌクレオシドとの親和性向上の分子 機構が解明されることを期待した。常温で、最高2.4 Å分解能データまでのX線回折データを測定した。単位 格子の体積、空間群、酵素の分子量から見積もって、非 対称単位には6量体の分子1つが含まれることが予想さ れた。そこで、野生型EB-APの6量体構造を探索モ デルとして、プログラムamoreを用いて分子置換法によ り解析を行った。rotation searchにおいては10~3 A分解能のデータを、translation searchにおいては1 0~4 Å分解能のデータを用いた。両サーチともに、正 解がトップピークとして現れた。分子を剛体として精密 化を行ったところ、R因子は37.3%に低下した。こ の後、QUANTAを用いたグラフィックス上での構造修正と X-PLORを用いた構造精密化を繰り返し行い、10~2. 4Å分解能において、R因子19.9%のモデルを得 た。実施例5と同様の方法で、5′-イノシン酸とG74 D/I153T変異型EB-APの結合モデルを作成し たところ、置換されたThr 153の側鎖のγ酸素原子 は、イノシンのリボースの水酸基と水素結合を形成する ことが予想された。また、もう一つ置換が施されたAsp 7 4 を含むループの揺らぎが、野生型に比べG 7 4 D/ I 153T変異型EB-APの方が大きくなっているこ とが、温度因子を比較することにより分かった。このル ープはイノシンの塩基と相互作用することが予想される が、揺らぎが大きくなったことにより塩基との結合がし やすくなった可能性が示唆される。

【0025】実施例7 野生型EB-APとモリブデン 酸との複合体(反応中間体アナログ)の結晶構造解析 EB-APの酵素反応において、まず初めに、リン酸モ ノエステル結合が切断され、リン酸基は活性残基のHis 189と共有結合を形成する。この状態の酵素分子を反 応中間体と呼ぶ。反応中間体は速やかに、水あるいはア ルコールによるアタックを受け、その結果、リン酸イオ ンが離脱する。水がアタックすればホスファターゼ活性 が発揮されることとなり、また、アルコールがアタック すればリン酸基転移活性が発揮されることになる。いず れにしても、反応中間体は不安定であり、その構造をX 線結晶構造解析により決定することは不可能である。し かし、リン酸の代わりにモリブデン酸がHis189に共 有結合したもの(反応中間体アナログ)は、水によるア タックを受けないので安定に存在する。リン酸基転移反 応においては、反応中間体にリン酸受容体が結合し、リ

ン酸モノエステル結合が形成される。したがって、ヌク レオシドとの結合様式を推定する目的においては、遊離 型構造よりも、反応中間体構造を用いる方が適切であ る。反応中間体とヌクレオシドとのドッキングスタディ を行う目的で、反応中間体アナログの結晶構造解析を行 った。常温にて、最高2.4Å分解能データまでのX線 回折データを測定した。単位格子の体積、空間群、酵素 の分子量から見積もって、非対称単位には6量体の半 分、すなわち、サブユニット3つが含まれることが予想 された。そこで、3回軸によってお互いが関係づけられ る3量体構造を作成し、分子置換法の探索モデルとし た。rotation searchにおいては10~3 Å分解能のデ ータを、translationsearchにおいては10~4Å分解 能のデータを用いた。両サーチともに、正解がトップピ ークとして現れた。分子を剛体として精密化を行ったと ころ、R因子は42.4%に低下した。この後、QUANTA を用いたグラフィックス上での構造修正とX-PLOR を用いた構造精密化を繰り返し行い、8~2.4 Å分解 能において、R因子22.3%のモデルを得た。非対称 単位には6量体の分子半分、つまりサブユニット3つが 含まれる。

【0026】実施例8 EB-AP反応中間体とイノシンの結合様式モデルの推測

コンピュータグラフィックス上、QUANTAを用いて、結合様式モデルを構築した(図3)。モリブデン酸はそのままリン酸に置き換えた。イノシンは、野生型EB-APと5′ーイノシン酸の結合様式モデルにおける5′ーイノシン酸のヌクレオシド部分近くに置いた。ただし、当然のことだが、イノシンはリン酸モノエステル結合を持たないので、5′ーイノシン酸をドッキングさせるよりも自由度が高い。したがって、イノシンがEB-APの分子表面に、より好ましい状態で結合するように、イノシンの位置の微調整を行い、結合様式モデルとした。以降の変異型酵素のデザインには、このモデルを用いることとした。

【0027】実施例9 ヌクレオシドとの親和性向上を 目指した変異型EB-APのデザイン

実施例8で構築したモデルによると、Ser 7 2の側鎖は、イノシンの塩基と相互作用する可能性が示唆された。この残基をフェニルアラニン、チロシン、トリプトファンといった芳香族アミノ酸に置換すると、芳香環とヌクレオシド塩基との間にπ-π相互作用が生じ、ヌクレオシドのEB-APに対する親和性が向上することが予測された。同様に、バリン、ロイシン、イソロイシンといった分岐鎖疎水性アミノ酸に置換すると、分岐鎖疎水基とヌクレオシド塩基との間にCH/π相互作用が生じ、また、グルタミン酸、アスパラギン酸といった負電荷を帯びたアミノ酸に置換すると、ヌクレオシド塩基の正電荷と静電的に引き合い、親和性の向上が見込まれた。そこで、ホスファターゼ活性よりもリン酸基転移活

性が相対的に高まっているG74D/I153T変異型 EB-APのリン酸基転移活性を更に高めるため、該変 異型EB-APのS72F, S72Y, S72W, S72 V, S72E, S72D変異体を作製することとした。 なお、S72を他のTS/酸に置換した変異体も作製することとした。 ちなみに、これら変異体は、3残基置換 変異型EB-APとなる。

【0028】実施例10 Ser72を他のアミノ酸へ置換した3残基置換変異型EB-APの作製

エシェリヒア・コリJM109で発現するための9種類の 変異型 EB-APを構築するために、G74D/ І 15 3 T変異型 E B - A P遺伝子を含むプラスミドpEPI 3 4 0を、 PCRを用いる部位特異的突然変異誘発法の鋳型 として使用した。なお、pEPI340は、プラスミドpEPI 305にG74D/I153Tの変異を加えたプラスミ ドである。しかして、これらプラスミドpEPI305及び pEPI340の塩基配列は、特開平10-201481号 公報の段落番号(0143)の表12に明記されてい る。また、プラスミドpEPI305をエシェリヒア・コリ JM109に保持させた株は、AJ13144と命名され、 通商産業省工業技術院生命工学工業技術研究所にFER M BP-5423として国際寄託されている〔上記公 開公報の段落番号(0105)~(0110)の記載参 照〕。変異はストラタジーン(Stratagene)社(アメリ カ)の「クイックチェンジ部位特異的突然変異誘発キッ ト (Quickchange Site-Directed Mutagenesis Kit) 」 を使用し、製造元のプロトコールに従って、各種変異型 酵素に対応するプライマー(図7、配列表の配列番号1 1~61)を用いて導入した。PCR反応の生成物を用 いて、エシェリヒア・コリXL-1を形質転換した。形 質転換細胞を、 100μ | / m | 0 p y y y y z z z zL寒天培地プレート上に塗沫し、37℃で16時間イン キュベートした。生成したコロニーを採取し、100μ I/mIのアンピシリンを含むL培地で一晩振とうしな がら培養した。培養液から遠心分離により菌体を回収 後、ファルマシア社(スウェーデン)のFlexiPrep Kit を使用し、製造元のプロトコールに従って、プラスミド の抽出を行った。各種3残基置換変異型EB-APをコ ードする塩基配列は、DNA配列分析によって確認した。なお、図7に示したプライマーセットの合成は(株)日本バイオサービスに委託した。

【0029】実施例11 変異型EB-APのリン酸基 転移活性及び反応速度定数の測定

各種3残基置換変異型EB-AP遺伝子を含むプラスミ ドを導入したエシェリヒア・コリJM109を100μl /mlのアンピシリンを含むL培地50mlに接種し、 37℃で16時間培養した。培養液から遠心分離で菌体 を集め、25mMリン酸バッファー(pH7.0)3ml に懸濁し、4℃で20分間超音波処理を行い破砕した。 処理液を遠心分離して不溶性画分を除き、無細胞抽出液 を調製した。各EB-AP3残基置換変異型酵素が発現 していることは、SDS-PAGEで確認した。発現量 は、全蛋白質の20%程度であった。無細胞抽出液のリ ン酸基転移活性は以下の条件で測定した。2mMイノシ ン、100mMピロリン酸ナトリウム、100mM酢酸 バッファー(pH4.0)、 100μ lの無細胞抽出液を 含む反応液(1ml)を、pH4、30℃で10分間イン キュベートした。1 Ν塩酸200μ Ι を加え反応を停止 させた後、遠心分離により沈殿を除き、生成した5'-イノシン酸を定量した。各種3残基置換変異型EB-A Pのリン酸基転移活性を、変異を導入する対象としたG 74D/I153T変異型EB-APを用いたときの 5'ーイノシン酸生成量を1とした相対活性で示した。 続いて、各種3残基置換変異型EB-APのリン酸基転 移反応におけるイノシンに対するKm値を以下の条件で 測定した。100mMピロリン酸ナトリウム、100m M酢酸バッファー(pH4.0)、10-100mMイノ シン、100μ | の無細胞抽出液を含む反応液(1m |) を p H 4、30℃で10分間インキュベートした。1 N 塩酸200μΙを加え反応を停止させた後、遠心分離に より沈殿を除き、生成した5′ーイノシン酸を定量し た。Hanes-WoolfプロットによりKm値を算出した。表 2に結果を示す。

[0030]

【表2】

	Km値	リン酸転移活性
S72F/G74D/I153T	20mM	2.80
S72Y/G74D/1153T	25mM	2.04
S72W/G74D/I153T	30mN	1.71
S72D/G74D/1153T	33mM	1.59
S72V/G74D/I153T	40mM	2.46
S72E/G74D/I153T	40mM	3.19
S72M/G74D/1153T	46mN	1.94
S72T/G74D/I153T	50mM	1.91
S72L/G74D/I153T	57mM	2. 24
S72R/G74D/I153T	59mM	1.99
S72Q/G74D/I153T	77mM	2.42
S72K/G74D/I153T	78mM	1.53
S72P/G74D/1153T	109mM	1.34
S72A/G74D/1153T	115mM	0.78
S72N/G74D/1153T	124mM	0.43
S72G/G74D/I153T	137mM	0.43
S72H/G74D/I153T	n. d.	n. d.
G74D/1153T	100mM	1.00
10残基置換変異型	40mM	1.44

【0031】実施例9にて、π-π相互作用、CH/π 相互作用、静電相互作用によりイノシンとの親和性が向 上するであろうと予測されたすべての変異体(S72F, S72Y, S72W, S72V, S72L, S72E, S7 2D)のイノシンに対するKm値が、変異導入しないG 74D/I153T変異型EB-APのものに比べて低 下し、イノシンに対する親和性が向上した。また、リン 酸基転移活性についても向上が見られた。特に、S72 Fを導入した変異体が、Km値、リン酸基転移活性、双 方において、改善が著しかった。フェニルアラニンの芳 香環とイノシン塩基が適当な位置関係でπーπ相互作用 し、親和性の向上が図られたものと推測される。また、 S72M, S72T, S72R, S72Q, S72K変異体 のKm値も低下した。これらアミノ酸残基とヌクレオシ ド塩基の間に疎水性相互作用、水素結合等何らかの好ま しい相互作用が生じたものと考えられる。ちなみに、S 72 | については、遺伝子を作製できなかった。また、 S72Cは、誤ったS-S結合を形成させる危険性があ るので、作製しなかった。なお、5'ーイノシン酸は、 高速液体クロマトグラフィー(HPLC)により、下記の条件 にて分析した。

カラム: Cosmosil 5 C 1 8-A R (4. 6×150 mm) ナカライテスク社製品

移動相:5 mM リン酸カリウムバッファー(pH2.8)/メタノール=95/5

流速: 1. 0ml/min

温度:室温

検出:UV245nm

【0032】実施例12 S72F/G74D/I15

3 T変異型 E B - A P遺伝子を導入したエシェリヒア・ コリJM109を用いた5′-イノシン酸の生産 G74D/I153T変異型、10残基置換変異型、及 びS72F/G74D/I153T変異型EB-AP遺 伝子を含むプラスミドを導入したエシェリヒア・コリJM 109をアンピシリン 100μ g/ml及びIPTG 1mMを含む L 培地 5 0 m l に接種し、3 7 ℃で 1 6 時 間培養した。ピロリン酸12g/d1、及びイノシン6 g/dlを酢酸バッファー(pH4.0)に溶解し、こ れに上記の各変異型EB-AP遺伝子を導入したエシェ リヒア・コリJM109の菌体を乾燥菌体重量で100m g/dlとなるように添加し、pHを4.0に維持しな がら、30℃で24時間反応を行った。生成した5′-イノシン酸の量を測定した結果を表3に示した。なお、 生成したイノシン酸は5'-イノシン酸のみで2'-イ ノシン酸及び3′ーイノシン酸の副生は全く認められな かった。G74D/I153T変異型EB-AP遺伝子 を含むプラスミドを導入したエシェリヒア・コリJM10 9を用いた反応では7.5g/dlの5'-イノシン酸 が生成蓄積したが、反応時間を伸ばしてもそれ以上蓄積 は増加しなかった。10残基置換変異型 EB-AP遺伝 子を含むプラスミドを導入したエシェリヒア・コリJM1 09を用いた反応では蓄積が向上し、12.1g/dl の5'-イノシン酸が生成蓄積した。立体構造に基づいて 設計し、構築したS72F/G74D/I153T変異 型EB-AP遺伝子を含むプラスミドを導入したエシェ リヒア・コリJM109を用いた反応では更に生産性が向 上し、13.2g/dlの5'-イノシン酸が生成蓄積 した。

[0033]

【表3】

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)			
G74D/I153T	7. 5			
10	12.1			
S72F/G74D/I153T	13. 2			

【0034】実施例13 L16W,S71W,S73W,E104F,E104W変異を導入した3残基置換変 異型EB-APのリン酸基転移活性及び反応速度定数の 測定

S72F変異が、 $\pi-\pi$ 相互作用によりイノシンとの親和性を向上させたと考えられるため、コンピューターグラフィックス上で、芳香環アミノ酸への置換によりイノシン塩基との $\pi-\pi$ 相互作用が図れる他のアミノ酸残基を探索した。その結果、L16W, S71W, S73W, E104F, E104W変異により置換された芳香環がイノシン塩基と相互作用する可能性が示唆された。そこで、これら5種の(G74D/I153T変異型EB-APをベースとした)3残基置換変異型EB-APを実施例10に記述した方法(各変異型酵素に対応するプラ

イマーは図8に示した。配列表の配列番号62~76)で作製し、実施例11に記述した方法でリン酸基転移活性及び反応速度定数を測定した。結果を表4に示す。リン酸基転移活性はいずれの変異型酵素においても低下したが、Km値はすべての変異型酵素で低下し、イノシンとの親和性が向上したことが示唆された。Leu16は、イノシンとの相互作用が確実視されるSer72から(Cα間距離で)10 Å離れているが、この程度離れていてもイノシンとの相互作用が可能であることが示された。なお、図8に示したプライマーセットの合成は、(株)日本バイオサービスに委託した。

【0035】 【表4】

	Kudi	リン酸基転移活性
L16W/G74D/I153T	33mM	0.21
S71W/G74D/I153T	75mM	0.26
S73W/G74D/I153T	29mM	0.77
E104F/G74D/I153T	61mM	0.65
E104W/G74D/I153T	67mM	0.26
G74D/1153T	100mM	1.00
10残基置換変異型酵素	40mM	1.44

【0036】実施例14 エンテロバクター・アエロゲネス(Enterobacter aerogenes)由来酸性ホスファターゼ (EA-AP)の変異型酵素遺伝子の作製と該遺伝子を 導入したエシェリヒア・コリJM109を用いた5′ーイノシン酸の生産

EB-APにおいてイノシンのリン酸基転移活性を向上させたS72F/G74D/I153Tの3つの変異に相同な変異をEA-APに導入することとした。EB-APとEA-APのアミノ酸配列をプログラムBLASTを用いて、アラインメントした結果を図9に示す。EB-APのSer72/Gly74/Ile153は、<math>EA-APにおいては、Ala70/Gly72/Ile151に対応するこ

とが示された。そこで、A70F/G72D/I151 T変異型EA-APを実施例10に記述した方法で作製した。変異型酵素遺伝子を含むプラスミドを導入したエシェリヒア・コリJM109を用いて、イノシンから5'ーイノシン酸の生産を実施例12に記述した方法で行った。結果を表5に示す。A70F/G72D/I151 T変異型EA-APは、S72F/G74D/I153 T変異型EB-APと同等の5'ーイノシン酸生産能を示した。

【0037】 【表5】

導入した変異型酵素遺伝子	生成イノシン酸 (g/dl)
EA-AP A72F/G74D/1153T	13. 1
EB-AP S72F/G74D/I153T	13. 2

【0038】なお、図9はEB-APとエンテロバクター・アエロゲネス由来酸性ホスファターゼ(EA-AP)のアミノ酸配列アラインメントをプログラムBLASTにより行った結果を示す図である。上段がEB-AP、下段がEA-APである。中段には、両者のアミノ酸残基が同一であればその残基名が、同一でなくても類似のアミノ酸残基であれば、+が表示される。EB-APの72番目の残基(Ser72)の位置を、[72]でマークした。EA-APで対応する残基はAIa70である。【0039】実施例15 モルガネラ・モルガニ由来酸性ホスファターゼ(MM-AP)由来G72D/I15

1 T変異型酵素の結晶化及び結晶構造解析 ハンギングドロップ法での蒸気拡散を利用してMM-A PのG72D/I151T二重変異体の結晶化を行った。当該蛋白 質溶液(濃度40mg/ml) と、25 (w/v%) のポリエチレン グリコール 1 O O O、25mM 硫安、25mM DTTを含む125mM クエン酸緩衝液 (pH4.8)を同量ずつ(各々5μI)、シ リコナイゼイションしたカバーグラス上に滴下混合し、 25 (w/v%) のポリエチレングリコール1000、25mM 硫安、25mM DTTを含む125mM クエン酸緩衝液 (pH4.8)50 0μ | を満たしたウエルの上に混合液滴が釣り下がるよう にかぶせ、20℃にて静置した。2、3日後に結晶が析出 し、1週間後には測定可能な大きさ(0.4×0.4×0.3mm 程度) に成長した。(株)リガクのX線回折装置R-AXIS Hcを用いて、X線回折データを収集し、結晶学的パラ メーターを決定した。空間群はP212121、格子定数はa= 90.64Å、b=119.74Å、c=136.14Åとなった。筑波・ 高エネルギー研究所シンクロトロン放射光施設BL-6B 上、100Kで、2.6Å分解能データまでの回折データを測 定した。単位格子の体積、空間群、酵素の分子量から見 積もって、非対称単位には6量体の分子1つが含まれる ことが予想された。そこで、野生型 EB-APの6量体 構造を探索モデルとして、プログラムamoreを用いて分 子置換法により解析を行った。rotation searchにおい ては10~3 Å分解能のデータを、translation search においては10~4Å分解能のデータを用いた。両サー チともに、正解がトップピークとして現れた。分子を剛 体として精密化を行った後、QUANTAを用いたグラフィッ クス上での構造修正とX-PLORを用いた構造精密化を繰り 返し行い、10~2.6Å分解能において、R因子0.197の モデルを得た。図1に示した5つの活性残基(Lys1 13、Arg120、His148、Arg181、H is 187)の $C\alpha$ 原子間の距離を表6に示した。EB -APの類縁酵素であるMM-APにおいて、活性残基間のすべての原子間距離が、EB-APの立体構造から規定した範囲におさまっていることが確認された。

[0040]

【表6】

		G74D/1153T 変異型MA-AP	下限	上限
Lys113	Arg120	11.3A	10.4Å	12.6Å
	His148	12.6Å	11.3Å	13. 8Å
	Arg181	16.3Å	14.5Å	17.4Å
	His187	12.5Å	10.7Å	13.6A
Arg120	His148	14.0Å	12. 2Å	15. 2Å
	Arg181	10. 9Å	9.4Å	11.8Å
	His187	6.1Å	4.5Å	6.7Å
His148	Arg18i	8. 9 A	6.7Å	9.8Å
	His187	10. 2Å	8.8A	11.0Å
Arg181	His187	5. 4Å	4.5Å	6.8A

【0041】実施例16 A72F、A72E変異を導入した10残基置換変異型EB-APの作製、及びリン酸転移活性と反応速度定数の測定

実施例11において、最もKm値を低下させたS72F と最も活性を高めたS72E変異を、10残基置換変異 型EB-APに導入した。10残基置換変異型EB-A Pにおいては、Ser72がAlaに置換されているの で、実際には、A72FとA72E変異を導入すること となる。野生型 EB-APを基準とすると、双方とも1 0残基が置換されている。これら2種の変異体を実施例 10に記述した方法(各変異型酵素に対応するプライマ ーは図46に示した。配列表の配列番号77~82)で 作製した。PCRを用いる部位特異的突然変異誘発法の 鋳型としては、10残基置換変異型EB-AP遺伝子を 含むプラスミドpEMP370 (特開平9-37785号公報、実施例 19)を使用した。更に、実施例11に記述した方法で リン酸転移活性及び反応速度定数を測定した。結果を表 7に示す。リン酸転移活性は、G74D/I153T変 異型EB-APを用いたときの5′-イノシン酸生成量 を1とした相対活性で示した。いずれの変異型酵素にお いても、Km値は顕著に低下した。リン酸転移活性につ いては、A72F変異により低下したのに対し、A72 E変異により上昇した。

[0042]

【表7】

	Km値	リン酸転移活性
A72F/10残基置換変異型酵素	9mM	0.11
A72E/10残基置換変異型酵素	15mM	2.30
10残基置換変異型酵素	40mM	1.44

【0043】実施例17 A72F/10残基置換変異型EB-AP及びA72E/10残基置換変異型遺伝子を導入したエシェリヒア・コリJM109を用いた5′-

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A72F/10残基置換変異型EB-AP及びA72E/10残基置換変異型遺伝子を含むプラスミドを導入し

たエシェリヒア・コリ J M 109を用いての 5′ ーイノシン酸の生産実験を実施例 1 2 に記述した方法で行った。 結果を表 8 に示す。両変異体とも 5′ ーイノシン酸の蓄 積量が増加した。 【0044】 【表8】

導入した変異型酵素遺伝子	生成イノシン酸(g/dl)
A72F/10残基置換変異型	13.9
A72E/10残基置換変異型	13.9
10残基置換変異型	12.1

I103D変異により、置換されたAspがイノシン塩基と静電相互作用をすること、また、T153N変異により、置換されたAsnがリボースの水酸基と水素結合を形成することが図3のモデルにより示唆された。そこで、G74D/I153T変異型EB-APにこれらの残基を導入し、I103D/G74D/I153T変異型EB-APとG74D/I153N変異型EB-APを実施例10に記述した方法(各変異型酵素に対応する

プライマーは図47に示した。配列表の配列番号83~88)で作製することとした。更に、実施例11に記述した方法でリン酸転移活性及び反応速度定数を測定した。結果を表9に示す。リン酸転移活性は、G74D/1153T変異型EB-APを用いたときの5′-イノシン酸生成量を1とした相対活性で示した。両変異体ともに、リン酸基転移活性は低下したが、Km値は低下し、イノシンとの親和性が向上したことが示唆された。

【0046】 【表9】

 Km値
 リン酸転移活性

 I103D/G74D/I153T
 51mM
 0.09

 G74D/I153N
 38mM
 0.18

 G74D/I153T
 100mM
 1.00

【0047】実施例19 Leu140をPhe、Glu、Lysに置換した変異型EB-APの作製、及びリン酸転移活性と反応速度定数の測定

Leu140は、Ser72から10Å以上離れているが、反応中間体アナログの立体構造において、リン酸結合部位の直近に位置する。したがって、この残基を置換すれば、反応中間体におけるリン酸結合部位周辺の構造が変化し、ひいては、ヌクレオシド結合部位の構造と揺らぎにも影響が及ぶものと考えられた。この残基を、よりかさ高いPhe、正電荷を有するLys、負電荷を有するGIu、に置換すれば、ヌクレオシドとの親和性が変化するのではないかと期待される。変異は、実施例16においてリン酸転移活性が高かったA72E/10残基置換体

に導入することとした。これら3種の変異体を実施例10に記述した方法(各変異型酵素に対応するプライマーは図48に示した。配列表の配列番号89~97)で作製した。PCRを用いる部位特異的突然変異誘発法の鋳型としては、A72E/10残基置換変異型EB-AP遺伝子を含むプラスミドを使用した。更に、実施例11に記述した方法でリン酸転移活性及び反応速度定数を測定した。結果を表10に示す。リン酸転移活性は、G74D/I153T変異型EB-APを用いたときの5′-イノシン酸生成量を1とした相対活性で示した。

[0048] [表10]

	Km値	リン酸転移活性・
A72E/L140F/10残基置換変異型酵素	9mM	1.66
A72E/L140K/10残基置換変異型酵素	78mM	0.07
A72E/L140E/10残基置換変異型酵素	322mM	0.16
A72E/10残基置換変異型酵素	15mM	2. 30

【0049】L140Fを導入した変異体は、Km値が低下した。逆に、L140K及びL140E変異は、Kmを大幅に上昇させた。

【0050】実施例20 エンテロバクター・アエロゲネス IFO 12010 由来野生型酸性ホスファターゼの精製とN末端アミノ酸配列の決定

特開平10-201481号公報の実施例24記載のエシェリヒア

・コリJM109/pENP110の培養菌体からエンテロバクター

・アエロゲネスIF012010由来の酸性ホスファターゼを精製してN末端アミノ酸配列を決定し、成熟蛋白質のアミノ酸配列を決定した。エシェリヒア・コリ JM109/pENP1 10はエンテロバクター・アエロゲネスIF012010由来の酸性ホスファターゼ遺伝子をエシェリヒア・コリJM109株に導入した菌で、該酸性ホスファターゼを生産する。該

酸性ホスファターゼ遺伝子の塩基配列より予想される前 駆体蛋白質のアミノ酸配列は配列表の配列番号10に示 される配列である。ペプトン 1 g/dl、酵母エキス0.5g/d | I及び食塩1g/dlを含有する栄養培地 (pH7.0) 50mlを50 0ml坂口フラスコに入れ、120℃にて20分間加熱殺菌し た。これにエシェリヒア・コリ JM109/pENP110を一白金 耳接種し、30℃で16時間振とう培養した。培養液から遠 心分離により菌体を回収した菌体を100 mlの100mMリ ン酸カリウムバッファー (pH7.0) に懸濁し、4℃で20 分間超音波処理を行い菌体を破砕した。処理液を遠心分 離して不溶性画分を除き、無細胞抽出液を調製した。こ の無細胞抽出液に30%飽和となるように硫酸アンモニウ ムを添加した。遠心分離により生成した沈殿を除去した 後、上清液に60%飽和となるように硫酸アンモニウムを 追加添加した。生成した沈殿を遠心分離により回収し、 100mMリン酸カリウムバッファーに溶解した。この粗酵 素液を100mMリン酸カリウムバッファー(pH7.0)500ml に対し3回透析した後、20mMリン酸カリウムバッファー (pH7.0) で平衡化したDEAE-トヨパール650Mカラム (φ 3.0×10.0cm) にチャージし、20mMリン酸カリウムバッ ファー (pH7.0) で洗浄した。リン酸転移活性は素通り 画分にあったので、当該画分を回収した。 この活性画 分に35%飽和となるように硫酸アンモニウムを添加し、 これを35%飽和硫酸アンモニウムを含む20mMリン酸カリ ウムバッファー (pH7.0) で平衡化したブチルトヨパー ルカラム (φ3.0×7.0cm) に吸着させた。これを35%飽 和から20%飽和リン酸カリウムバッファー (pH7.0) の 直線的な濃度勾配で溶出した。活性画分を集め、10mMリ ン酸カリウムバッファー (pH6.0) 1 L に対し透析した 後、10mMリン酸カリウムバッファー (pH6.0) で平衡化 したCM-トヨパール カラム (φ3.0×7.0cm) に吸着させ た。これを0mMから300mM 塩化カリウムを含むリン酸カ リウムバッファー (pH6.0) の直線的な濃度勾配で溶出 した。この活性画分を集めた。以上の操作によって、リ ン酸転移活性を示す酵素を無細胞抽出液より最終的に約 16%の回収率で約5倍に精製した。この酵素標品は、S DS-ポリアクリルアミド電気泳動において均一であっ た。本精製酵素をDITC メンブレン〔ミリゲン/バ イオサーチ (Milligen/Biosearch) 社製) に吸着させ、 Prosequencer 6625 (ミリゲン/バイオサーチ社製) を 用いてN末端のアミノ酸配列を決定したところ配列表の 配列番号98に示した5残基のN末端のアミノ酸配列が 決定された。精製酵素のN末端は配列表の配列番号10 の配列の21番目のアラニン残基から開始していたため、 配列表の配列番号10に示されるアミノ酸配列は前駆体 蛋白質の配列であり、1番目のメチオニン残基から20番 目のフェニルアラニン残基までのペプチドは翻訳後に除 去されるものと考えられた。この結果より成熟体蛋白質 のアミノ酸配列は配列表の配列番号10に示される配列 中、アミノ酸番号1~228に示される配列であると考

えられた。

【0051】実施例21 エンテロバクター・アエロゲ ネス IFO 12010 由来新規変異型酸性ホスファターゼ遺 伝子のプロモーター配列の改変による酵素の高発現 エンテロバクター・アエロゲネス IFO 12010由来変異型 酸性ホスファターゼをコードする遺伝子のプロモーター 配列部分に遺伝子工学的手法によって部位特異的変異を 導入し、酵素発現量の増加した変異型酸性ホスファター ゼをコードする遺伝子を構築した。変異を導入する遺伝 子は特願平12-189226号明細書の実施例3にて構築したプ ラスミドpENP170を用いた。本プラスミドはエンテロバ クター・アエロゲネス IFO 12010由来変異型酸性ホスフ アターゼをコードする遺伝子を含む制限酵素Sallと 制限酵素Kpnlで切り出される1.6kbpDNA断片を、 Sall及びKpnlで切断したpUC19(宝酒造社製) に連結したプラスミドDNAであり、pENP170中のSa II-KpnI 1.6kbpDNA断片の塩基配列は配列表 の配列番号9に示される配列である。プラスミドDNA への変異導入はストラタジーン社製のクイックチェンジ 部位特異的突然変異誘発キット (Quick Change sitedirected mutagenesis kit)を用いた。DNA合成装 置(アプライドバイオシステム社製モデル394)を用い て合成した変異導入用オリゴヌクレオチドMUT170(配列 表の配列番号99)、MUT171(配列表の配列番号10 O)、及び鋳型としてpENP170を用いてストラタジーン 社のプロトコールに従って変異を導入した。得られたプ ラスミドDNAを用いて常法によりエシェリヒア・コリ JM109(宝酒造社製)を形質転換した。これを100 μ g/ mlのアンピシリンを含むL寒天培地上にプレーテイング し、形質転換体を得た。形質転換体よりアルカリ溶菌法 によりプラスミドを調製し、塩基配列の決定を行い、目 的の塩基が置換されていることを確認した。塩基配列の 決定は Tag DyeDeoxy Terminator Cycle Seguencing K it (アプライドバイオケミカル社製)を用い、サンガ ーらの方法〔ジャーナル オブ モレキュー バイオロジ ー (J. Mol. Biol.)、第143巻、第161頁(1980)〕に 従って行った。このようにしてエンテロバクター・アエ ロゲネス IFO 12010由来推定酸性ホスファターゼの上流 に位置する推定プロモーター配列の-10領域の塩基配 列がAAAATからエシェリヒア・コリのlacプロモーター と同じTATAATという塩基配列に変異した変異型遺伝子を コードする変異型遺伝子を構築した。この変異型遺伝子 を含むプラスミドをpENP180と命名した。

【0052】エシェリヒア・コリ JM109/pENP170及 びプロモーター配列の-10領域を改変した遺伝子を導入したエシェリヒア・コリJM109/pENP180をアンピシリン100 μ g/mlを含むL培地50m I、及び IPTG1mM を添加したアンピシリン100 μ g/mlを含むL培地50m Iにそれぞれ接種し、37℃で16時間培養した。それぞれの菌の培養液から遠心分離により菌体を集め、生理食塩水

で1回洗浄した。ピロリン酸15g/dI、及びイノシン、8g/dlを100mM酢酸バッファー(pH4.0)に溶解し、これにそれぞれの菌体を乾燥菌体重量で100mg/dIとなるように添加し、pHを4.0に維持しながら、30℃で1時間反応させた。生成した5′ーイノシン酸の量を表 1 1に示した。イノシン及び5′ーイノシン酸は、高速液体クロマトグラフィー(HPLC)により、下記の条件にて分析した。

カラム:Cosmosil 5 C 18-AR (4.6×150mm) 〔ナカライテスク社製品〕

移動相:5mM リン酸カリウムバッファー(pH 2.8)/メ

タノール = 95/5

流速:1.0ml/min 温度:室温 検出:UV245nm

エシェリヒア・コリJM109/pENP170ではIPTG無添加では活性が低かったが、エシェリヒア・コリJM109/pEN P180はIPTGを添加しなくても高い活性を示した。また、エシェリヒア・コリJM109/pENP180はIPTGを添加することでさらに高い活性を発現し、プロモーター領域の改変が有効であることが示された。

【0053】 【表11】

歯 株	IPTG	生成 5'ーイノシン酸(g/dl)
エシェリヒア・コリ	無添加	0.73
JN109/pENP170	1 mM 添加	3.09
エシェリヒア・コリ	無添加	2.86
JM109/pENP180	1 mM添加	3. 37

【0054】実施例22 ヌクレオシドに対する親和性 の向上したエンテロバクター・アエロゲネス IFO 12010 由来新規変異型酸性ホスファターゼ遺伝子の構築 実施例21にて構築したエンテロバクター・アエロゲネ ス IFO 12010由来変異型酸性ホスファターゼ遺伝子に遺 伝子工学的手法によって部位特異的変異を導入し、ヌク レオシド、特にグアノシンに対する親和性が向上した変 異型酸性ホスファターゼをコードする遺伝子を作製し た。アミノ酸残基の置換はエシェリヒア・ブラッタエ酵 素の立体構造解析に基づいてヌクレオシドとの親和性向 上に寄与すると同定されたアミノ酸残基の置換を組合せ て導入した。プラスミドDNAへの変異導入はストラタ ジーン社製のクイックチェンジ部位特異的突然変異誘発 キット (Quick Change site-directed mutagenesis kit)を用いた。DNA合成装置(アプライドバイオシ ステム社製モデル394) を用いてMUT180 (配列表の配列 番号101) からMUT521(配列表の配列番号120) ま での20種類の変異導入用オリゴヌクレオチドを合成し た。最初の鋳型としてpENP170、また変異導入用オリゴ ヌクレオチドとしてMUT180、MUT181を用いてストラタジ ーン社のプロトコールに従って変異を導入した。得られ たプラスミドDNAを用いて常法によりエシェリヒア・ コリJM109(宝酒造社製)を形質転換した。これを100 μq/mlのアンピシリンを含むL寒天培地上にプレーテイ ングし、形質転換体を得た。形質転換体よりアルカリ溶 菌法によりプラスミドを調製し、塩基配列の決定を行 い、目的の塩基が置換されていることを確認した。塩基 配列の決定は Tag DyeDeoxy Terminator Cycle Seguen cing Kit (アプライドバイオケミカル社製)を用い、 サンガーらの方法(前出の学会誌)に従って行った。こ のようにして153番目のスレオニン残基(ACC)がセリン

残基(TCC)に置換した変異型酸性ホスファターゼをコードする遺伝子を構築し、この変異型遺伝子を含むプラスミドをpENP200と命名した。変異を導入したプラスミドを新しい鋳型として同様の操作を繰り返し、累加的に部位特異的変異を導入した。形質転換体よりアルカリ溶菌法によりプラスミドを調製し、塩基配列の決定を行い、目的の塩基が置換されていることを確認した。作成した変異型酸性ホスファターゼをコードする変異型酵素遺伝子と変異部位を表12に示した。なお変異部位のアミノ酸残基は配列表の配列番号10に示した成熟蛋白質のアミノ酸配列中のアミノ酸残基を示している。

【0055】それぞれの変異型酸性ホスファターゼ遺伝子を含むプラスミドを導入したエシェリヒア・コリ J M109/pENP180、エシェリヒア・コリ J M109/pENP320、エシェリヒア・コリ J M109/pENP340、エシェリヒア・コリ J M109/pENP340、エシェリヒア・コリ J M109/pENP520をアンピシリン100 μg/ml 及び I P T G 1 m Mを含むし培地50m I に接種し、37℃で16時間培養した。菌体を50m I の100m M リン酸バッファー(pH7.0)に懸濁し、4℃で20分間超音波処理を行い菌体を破砕した。それぞれの菌の培養液から遠心分離により菌体を集め、生理食塩水で1回洗浄した。処理液を遠心分離して不溶性画分を除き、無細胞抽出液を調製した。それぞれの無細胞抽出液を用いてリン酸転移反応におけるイノシンとグアノシンに対するKm値を測定した。

【0056】ヌクレオシドへのリン酸転移活性の測定は、イノシン及びグアノシンを基質として次の条件で行った。各種濃度のイノシン又はグアノシン、ピロリン酸ナトリウム $100\,\mu$ mol/ml、酢酸ナトリウム緩衝液(pH4.0) $100\,\mu$ mol/ml及び酵素を含む反応液(1ml)でpH4.0、

30℃で10分反応を行った。2N塩酸200 μ I を添加して反応を停止した後、遠心分離により沈殿を除き、リン酸転移反応により生成した5′ーイノシン酸又は5′ーグアニル酸を定量した。イノシン、グアノシン、5′ーイノシン酸及び5′ーグアニル酸は、高速液体クロマトグラフィー(HPLC)により、実施例21と同じ条件にて分析した。上記の組成の反応条件においてイノシン又はグアノシンの濃度を変化させてリン酸転移活性を測定し、Hanes-Woolfプロット〔ザ バイオケミカル ジャーナル(Biochem. J.)、第26巻、第1406頁(1932)〕により

リン酸転移反応におけるイノシン及びグアノシンの速度 定数を求めた。その結果を表 13~表 16に示した。実 施例で作成した変異型酵素のKm値はグアノシンに対する Km値が顕著に低下し、グアノシンに対する親和性が向 上していることが明らかになった。またpENP520にコー ドされる変異型酵素以外の4種類の変異型酵素はイノシ ンに対するKm値も非常に低下していた。

【0057】 【表12】

配列の名称	配列番号		長さ	配 列
MUT170	99	センス	30	5'-CTT ACA GAT GAC TAT AAT GTG ACT AAA AAC
MUT171	100	アンチセンス	30	5'-GTT TTT AGT CAC ATT ATA GTC ATC TGT AAG
MUT180	101	センス	33	5'-TCT ACC GGT TGG GCA TCC GCG CTG GTA CTG GCG
MUT181	102	アンチセンス	33	5'-CGC CAG TAC CAG CGC GGA TGC CCA ACC GGT AGA
MUT300	103	センス	33	5'-TCC GGC CAT ACC TCT TCC GGT TGG GCA TCC GCG
MUT301	104	アンチセンス	33	5'-CGC GGA TGC CCA ACC GGA AGA GGT ATG GCC GGA
MUT310	105	センス	33	5'-GAT GCT GAC CTG GCC GTT GGC GAC GTC GCG AAT
MUT311	106	アンチセンス	33	5'-ATT CGC GAC GTC GCC AAC GGC CAG GTC AGC ATC
MUT320	107	センス	33	5'-CTG ACA AAT ATG ATT CTG GAT GCC GGC GAT CTG
MUT321	108	アンチセンス	33	5'-CAG ATC GCC GGC ATC CAG AAT CAT ATT TGT CAG
MUT330	109	センス	33	5'-GAT GCT GAC CTG GCC ATG GGC GAC GTC GCG AAT
MUT331	110	アンチセンス	33	5'-ATT CGC GAC GTC GCC CAT GGC CAG GTC AGC ATC
MUT340	111	センス	33	5'-CTG ACA AAT ATG ATT CAG GAT GCC GGC GAT CTG
MUT341	112	アンチセンス	33	5'-CAG ATC GCC GGC ATC CTG AAT CAT ATT TGT CAG
MUT400	113	センス	33	5'-TCC GGC CAT ACC TCT GCT GGT TGG GCA TCC GCG
MUT401	114	アンチセンス	33	5'-CGC GGA TGC CCA ACC AGC AGA GGT ATG GCC GGA
MUT500	115	センス	33	5'-TCC GGC CAT ACC TCT GGC GGT TGG GCA TCC GCG
MUT501	116	アンチセンス	33	5'-CGC GGA TGC CCA ACC GCC AGA GGT ATG GCC GGA
MUT510	117	センス	33	5'-GAT GCT GAC CTG GCC GAA GGC GAC GTC GCG AAT
MUT511	118	アンチセンス	33	5'-ATT CGC GAC GTC GCC TTC GGC CAG GTC AGC ATC
MUT520	119	センス	33	5'-GAT GCT GAC CTG GCC AAA GGC GAC GTC GCG AAT
MUT521	120	アンチセンス	33	5'-ATT CGC GAC GTC GCC TTT GGC CAG GTC AGC ATC

[0058]

プラスミド名	変異を導入し	変異導入に用い	変異点及びアミノ酸置換
	たプラスミド	たプライマー	交換が及びラミア設固接
pENP180	707777	707711	61L(CTG)→Q(CAG)
peni 100			
			63A(GCT)→Q(CAG)
ł		1	64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
Wiss and			151I(ATC)→T(ACC)
pENP200	pENP130	MUT180, MUT181	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			151T(ACC)→S(TCC)
pENP300	pENP200	MUT300, MUT301	61L(CTG)→Q(CAG)
		-	63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
	Ì		69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
1			151I(ATC)→T(ACC)
			149T(ACC)→S(TCC)
	•		151T(ACC)~>S(TCC)
pENP310	pENP300	MUT310, MUT311	61L(CTG)→Q(CAG)
		M01010, M01011	63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			$67N(AAC) \rightarrow D(GAC)$
			69S(AGC)→A(GCC)
			70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
	[151I(ATC)→T(ACC)
			149T(ACC)→S(TCC)
~PWD990	- Dilboro	100000000000000000000000000000000000000	151T(ACC)→S(TCC)
pENP320	pENP310	MUT320, MUT321	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)

[0059]

【表14】

1			69S(AGC)→A(GCC)
1			70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)
			$102E(GAG)\rightarrow L(CTG)$
			133T(ACC)→K(AAA)
}			134E(GAG)→D(GAC)
			149T(ACC)→S(TCC)
			151T(ACC)→S(TCC)
pENP330	pENP300	MUT330, MUT331	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
	***************************************		70A(GCC)→M(ATG)
			72G(GGC)→D(GAC)
Ì			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)-S(TCC)
			151T(ACC)→S(TCC)
pENP340	pENP330	MUT340, MUT341	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
	ł		70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)
			102E(GAG)→Q(CAG)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
	1		149T(ACC)→S(TCC)
			151T(ACC)→S(TCC)
pENP400	pENP 200	MUT400, MUT401	61L(CTG)→Q(CAG)
•		1.01.100,1001.401	63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA) 67N(AAC)→D(GAC)
			69S(AGC)→A(GCC) 72G(GGC)→D(GAC)
			1 ' ' ' '
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
	1		149T(ACC)→A(GCT)
DENP410	pENP400	MUT310. MUT311	151T(ACC)→S(TCC)
Post a LV	PLIN 400	muratu, murati	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
ż			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→V(GTT)
			72G(GGC)→D(GAC)

[0060]

【表15】

			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→A(GCT)
DWDEAA			$151T(ACC) \rightarrow S(TCC)$
pENP500	pENP200	MUT500, MUT501	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			72G(GGC)→D(GAC)
1			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→G(GGC)
			151T(ACC)-S(TCC)
pENP510	pENP500	MUT510, MUT511	61L(CTG)→Q(CAG)
			63A(GCT)→Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
i			69S(AGC)→A(GCC)
			70A(GCC)→E(GAA)
			72G(GGC)→D(GAC)
			133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→G(GGC)
			151T(ACC)→S(TCC)
pENP520	pENP500	MUT520, MUT521	61L(CTG)→Q(CAG)
			63A(GCT)-Q(CAG)
			64E(GAA)→A(GCA)
			67N(AAC)→D(GAC)
			69S(AGC)→A(GCC)
			70A(GCC)→K(AAA)
			72G(GGC)→D(GAC)
	, ,		133T(ACC)→K(AAA)
			134E(GAG)→D(GAC)
			149T(ACC)→G(GGC)
			151T(ACC)→S(TCC)

[0061]

【表16】

		イノシンを基	グアノシンに	グアノシンを
	する Km 値	質とした場合	対するに血値	基質とした時
	(mM)	の相対活性	(mM)	の相対活性
pENP180	4 0	1. 0	40	1. 0
pBNP320	1 9	1. 9	4. 6	1. 5
pBNP340	1 9	1. 4	5. 1	1. 3
pBNP410	18	1. 0	4. 9	0.70
pENP510	1 7	0.55	4. 0	0.39
pENP520	4 6	0.63	4. 4	0.21

【0062】実施例23 グアノシンに対する親和性の向上したエンテロバクター・アエロゲネス IFO 12010 由来新規変異型酸性ホスファターゼ遺伝子導入菌によるグアノシンのリン酸化反応

それぞれの変異型酸性ホスファターゼ遺伝子を含むプラスミドを導入したエシェリヒア・コリ JM109/pENP18 0、エシェリヒア・コリJM109/pENP320、エシェリヒア・コリJM109/pENP340、エシェリヒア・コリJM10 9/pENP410、エシェリヒア・コリJM109/pENP510、及びエシェリヒア・コリJM109/pENP520をアンピシリン

100 μg/mI及び I P T G 1m M を含む L 培地50m I に接種し、37℃で16時間培養した。ピロリン酸10g/dI、及び特開平12-189226号公報の実施例 1 と同様に調製した粉砕グアノシン6.6g/dIを100m M 酢酸バッファー(pH4.5)に溶解し、これにそれぞれの菌体を乾燥菌体重量で100mg/dIとなるように添加し、pHを4.5に維持しながら、35℃で12時間反応させた。生成した5′ーグアニル酸の量を表 1 7 に示した。表 1 7 に示すように変異型酵素を導入した菌はいずれも親株であるエシェリヒア・コリ J M 109 / pENP180に比べて生産性が向上し、高い収率で5′

ーグアニル酸を生成蓄積した。

【表17】

[0063]

菌株	生成 5'-グアニル酸 (g/dl)
エシェリヒア・コリ	9. 90
IM109/pENP180 .	9. 90
エシェリヒア・コリ	10.4
JM109/pENP320	10.4
エシェリヒア・コリ	10.2
JM109/pENP340	10. 2
エシェリヒア・コリ	11.1
JM109/pENP410	11.1
エシェリヒア・コリ	11.0
JN109/pENP610	11.0
エシェリヒア・コリ	10.5
JN109/pENP520	10.5

[0064]

【発明の効果】以上詳細に説明したように、本発明によれば、ヌクレオシド-5′-リン酸生産能が向上した変異型ヌクレオシド-5′-リン酸生産酵素、及びその製造方法が提供される。また、本発明によれば、ヌクレオシド-5′-リン酸の製造方法に有用な、前記の変異型

酵素をコードする遺伝子、該遺伝子を含む組換えDNA、該組換えDNAを保有する微生物が提供される。更に、X線結晶構造解析技術により蛋白質の新規立体構造の解明に成功した。

[0065]

【配列表】

<;110>; Ajinomoto Co., Inc. (味の素株式会社)

〈;120〉;変異型ヌクレオシドー5′ーリン酸生産酵素

<;130>; 整理番号 1-000804-1

<;141>; 2000-09-03

<;150>; JP 11-249545

<;151>; 1999-09-03

<;160>;

<;170>; PatentIn Ver. 2.0

[0066]

<;210>; 1

<;211>; 1225

<;212>; DNA

<;213>; Escherichia blattae

<;220>;

<;221>; CDS

<;222>; (331)..(1077)

<;400>; 1

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Met Lys Lys Arg Val Leu Ala Val

tgt ttt gcc gca ttg ttc tct tct cag gcc ctg gcg ctg gtc gct acc 402 Cys Phe Ala Ala Leu Phe Ser Ser Gln Ala Leu Ala Leu Val Ala Thr

0 15 2

ggc aac gac act acc acg aaa ccg gat ctc tac tac ctc aag aac agt 450 Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu Tyr Tyr Leu Lys Asn Ser 25 30 35 40

gaa	gcc	att	aac	agc	ctg	gcg	ctg	ttg	ccg	cca	cca	ccg	gcg	gtg	ggc	498
Glu	Ala	He	Asn	Ser	Leu	Ala	Leu	Leu	Pro	Pro	Pro	Pro	Ala	Val	Gly	
				45					50					55		
tcc	att	gcg	ttt	ctc	aac	gat	cag	gcc	atg	tat	gaa	cag	ggg	cgc	ctg	546
Ser	He	Ala	Phe	Leu	Asn	Asp	GIn	Ala	Met	Tyr	Glu	Gln	Gly	Arg	Leu	
			60					65					70			
_	_			-	_		_				_	_	gca		_	594
Leu	Arg		Thr	Glu	Arg	Gly	•	Leu	Ala	Ala	Glu	•	Ala	Asn	Leu	
		75					80					85				
_	_						-						ggt	_	_	642
Ser		ч	Gly	Val	Ala		Ala	Phe	Ser	ыу		Phe	Gly	Ser	Pro	
atc	90	a 22	222	~ 2.6	200	95	aea	eta	ent.	222	100	cta	200	224	ata.	690
		-		-	-	_		_				_	acc Thr		_	090
105	1111	uiu	Lys	vəh	110	110	nia	Leu	1113	115	Leu	Leu	1111	MOII	120	
	gag	gac	acc	aaa		cta	aca	acc	cac		aca	aaa	gat	cac		738
		_	-		_	_			-	-	_		Asp			,50
			,,,,	125			,,,,		130	20.		-,-		135	•,,	
atg	cqc	att	cqt	ccq	ttc	gcq	ttt	tat	ggg	qtc	tct	acc	tgt	aat	acc	786
_	_		-	-						_			Cys			
	J		140					145	·				150			
acc	gag	cag	gac	aaa	ctg	tcc	aaa	aat	ggc	tct	tat	ccg	tcc	ggg	cat	834
Thr	Glu	GIn	Asp	Lys	Leu	Ser	Lys	Asn	Gly	Ser	Tyr	Pro	Ser	Gly	His	
		155					160					165				
acc	tct	atc	ggc	tgg	gct	act	gcg	ctg	gtg	ctg	gca	gag	atc	aac	cct	882
Thr	Ser	He	Gly	Trp	Ala	Thr	Ala	Leu	Val	Leu	Ala	Glu	He	Asn	Pro	
	170					175					180					
-	-	_				_		-			-	-	ggc	-		930
	Arg	GIn	Asn	Glu		Leu	Lys	Arg	Gly		Glu	l.eu	Gly	Gln		
185					190					195					200	070
			-					_	_	-		-	gcc			978
arg	vai	116	Cys	•	ıyr	HIS	ırp	GIN		ASP	vai	ASP	Ala		Arg	
ata	ata	993	tet	205	att	ata	aca	266	210	cat	266	226	ccg	215	ttc	1026
-				_	_				-				Pro			1020
vai	Vai	uly	220	nia	Vai	vai	Ala	225	Leu	1113	1111	MOII	230	Ala	ille	
caa	can	caa		can	aaa	aca	ลลต		กลล	ttc	acc	can	cat	can	aan	1074
_	_	_	_	_			_	_	_		_	_	His	_	_	
• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	235	LCu		_,,	,,,,	240	,u		, ,,,	,,,,	245		0	-,5	
aaa	taa		qac :	tacc	qcct	ta c	cttq	cagg	g cg	gtag	taat		cact	ggc		1127
Lys		,	,	•	,	J	,	,,,	, ,	, ,	,,			,,		
	gatte	cgc ·	tatte	ccca	ca g	taat	aatg	a cg	gtat	atga	ttt	tgtg	caa	cgaa	aaggtt	1187
gtg	tcac	gcc a	acag	ctta	ta a	gatc	atgt	g cc	gtta	ac				_	- •	1225
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[0067]

<;400>; 2

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10

1

[0068]

5

15

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		15					20					25				
acc	acc	aag	ccg	gat	tta	tat	tat	ctg	aaa	aat	gaa	cag	gct	atc	gac	447
Thr	Thr	Lys	Pro	Asp	Leu	Tyr	Tyr	Leu	Lys	Asn	Glu	GIn	Ala	He	Asp	
	30					35					40					
agc	ctg	aaa	ctg	tta	ccg	cca	ccg	ccg	gaa	gtc	ggc	agt	att	cag	ttt	495
Ser	Leu	Lys	Leu	Leu	Pro	Pro	Pro	Pro	Glu	Val	Gly	Ser	He	Gln	Phe	
45					50					55					60	
	aat	_	-	-	-					_	-	-	-			543
Leu	Asn	Asp	GIn		Met	Tyr	Glu	Lys	•	Arg	Met	Leu	Arg		Thr	
				65					70					75		
	cgc			-	-	-	_	_	_		-	-				591
Glu	Arg	Gly	•	GIn	Ala	Gln	Ala	-	Ala	Asp	Leu	Ala		Gly	Gly	
_			80					85					90			
	gca		-				_				_			_		639
vai	Ala		на	Pne	Ser	ыу		rne	ыу	ıyr	Pro		inr	GIU	Lys	
	+-+	95	~~~		***		100	~+~		t		105		an+	~~~	607
-	tct	-		_			_	_			_			-	_	687
wsh	Ser 110	F10	diu	Leu	1 9 1	115	Leu	Leu	1111	ASII	120	116	uiu	ASP	Ala	
aat	gat	ctt	acc	200	cac		acc	222	ass	cat		ata	cac	atc	caa	735
	Asp		-		_		-		-				_			133
125	пэр	LCu	ліа	1111	130	Jei	Alu	Lys	Giu	135	ıyı	me c	ліу	110	140	
	ttt	aca	ttt	tac		aca	ตลล	acc	tat		acc	aaa	gat	can		783
_	Phe						-		-				-	-		
				145	,	••••			150		,	-,-		155		
aaa	ctc	tcc	acc	aac	gga	tct	tac	ccg	tca	ggt	cat	acg	tct	atc	ggc	831
	Leu							_				_				
			160		•		•	165		•			170		•	
tgg	gca	acc	gca	ctg	gtg	ctg	gcg	gaa	gtg	aac	ccg	gca	aat	cag	gat	879
Trp	Ala	Thr	Ala	Leu	Val	Leu	Ala	Glu	Val	Asn	Pro	Ala	Asn	GIn	Asp	
		175					180					185				
gcg	att	ctg	gaa	cgg	ggt	tat	cag	ctc	gga	cag	agc	cgg	gtg	att	tgc	927
Ala	He	Leu	Glu	Arg	Gly	Tyr	Gln	Leu	Gly	GIn	Ser	Arg	Val	He	Cys	
	190					195					200					
ggc	tat	cac	tgg	cag	agt	gat	gtg	gat	gcc	gcg	cgg	att	gtc	ggt	tca	975
Gly	Tyr	His	Trp	GIn	Ser	Asp	Val	Asp	Ala	Ala	Arg	He	Val	Gly	Ser	
205					210					215					220	
	gct							_	_	-		_	-	_		1023
Ala	Ala	Val	Ala		Leu	His	Ser	Asp		Ala	Phe	GIn	Ala		Leu	
				225					230					235		
	aaa	_		_	_		-				_		taa	aagca	agt	1072
Ala	Lys	Ala		GIn	Glu	Phe	Ala		Lys	Ser	GIn	Lys				
240 245 gatatctggt cagggcagtg caatatctgc cctgaaatcc ctgtttattc ccacatccag																
							-		-		_				-	
cggtcttccc gatcccagcc ttttgttttc atgcagctgt agaaat												_				
tcttcattca catccatcac ataactttcc gttaccggtg tctgctcttt gtaggttttg ctgttaccgc agtcatcgtc ttttttgcag cgtttctcca catcccgcat cacactgcgc												_				
-		_	-	_					tttc	tcca	cat	cccg	cat	caca	tgcgc	
rgad	gcaad	ITT (catt	tttc	aC C	ggata	aaag	t tt								1344

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                                 25
Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala IIe Asp Ser Leu Lys Leu
Leu Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
                     70
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                                     90
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
                                105
Leu Tyr Lys Leu Leu Thr Asn Met lle Glu Asp Ala Gly Asp Leu Ala
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
                        135
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
145
                                        155
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                                    170
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala IIe Leu Glu
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
                            200
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
                    230
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                245
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<;211>; 991
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<;213>; Salmonella typhimurium
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agtgagtctt t atg aaa agt cgt tat tta gta ttt ttt cta cca ctg atc 170
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Met Lys Ser Arg Tyr Leu Val Phe Phe Leu Pro Leu IIe

10

5

1

[0070]

gta	gct	aaa	tat	aca	tca	gca	gaa	aca	gtg	caa	ccc	ttt	cat	tct	cct	218
Val	Ala	Lys	Tyr	Thr	Ser	Ala	Glu	Thr	Val	Gln	Pro	Phe	His	Ser	Pro	
	15					20					25					
gaa	gaa	tca	gtg	aac	agt	cag	ttc	tac	tta	cca	cca	ccg	cca	ggt	aat	266
Glu	Glu	Ser	Val	Asn	Ser	GIn	Phe	Tyr	Leu	Pro	Pro	Pro	Pro	Gly	Asn	
30					35					40					45	
gat	gat	ccg	gct	tac	cgc	tat	gat	aag	gag	gct	tat	ttt	aag	ggc	tat	314
					_		_			_			_	Gly		
•	•			50	-	•	•	•	55		•			60	•	
qcq	ata	aag	ggt	tcc	ccq	cqa	tgg	aaa	caa	qct	gct	gag	gat	gca	gat	362
Ala	He	Lys	Gly	Ser	Pro	Arg	Trp	Lys	Gln	Ala	Ala	Glu	Asp	Αla	Asp	
		•	65			_	•	70					75		•	
gta	agc	gtg	gaa	aat	ata	qcc	aga	ata	ttc	tcq	cca	qta	gtg	ggt	gct	410
~	-		•			-	-			-		-		Gly	_	
		80					85					90		•		
aaa	att	aac	ccc	aaa	gat	acq	cca	gaa	acc	tqq	aat	atq	tta	aag	aat	458
Lys	He	Asn	Pro	Lys	Asp	Thr	Pro	Glu	Thr	Trp	Asn	Met	Leu	Lys	Asn	
Ť	95			•	•	100				•	105					
ctt	ctg	aca	atg	ggc	ggc	tac	tac	gct	act	gct	tcg	gca	aaa	aaa	tat	506
	-		-					_		-	_	-		Lys		
110				·	115	•				120			•	•	125	
tat	atg	cgt	acc	cgc	ссс	ttt	gtc	tta	ttt	aat	cat	tcc	acc	tgc	cgt	554
Tyr	Met	Arg	Thr	Arg	Pro	Phe	Val	Leu	Phe	Asn	His	Ser	Thr	Cys	Arg	
				130					135					140	-	
cct	gaa	gat	gag	aat	act	ttg	cga	aaa	aat	ggc	tct	tac	cct	tcc	ggg	602
Pro	Glu	Asp	Glu	Asn	Thr	Leu	Arg	Lys	Asn	Gly	Ser	Tyr	Pro	Ser	Gly	
			145					150					155			
cat	act	gct	tat	ggt	aca	ctt	ctg	gca	tta	gta	tta	tcc	gag	gcc	aga	650
His	Thr	Ala	Tyr	Gly	Thr	Leu	Leu	Ala	Leu	Val	Leu	Ser	Glu	Ala	Arg	
		160					165					170				
ccg	gaa	cgc	gcg	cag	gag	ctc	gcc	aga	cgc	gga	tgg	gag	ttc	ggg	caa	698
Pro	Glu	Arg	Ala	Gln	Glu	Leu	Ala	Arg	Arg	Gly	Trp	Glu	Phe	Gly	Gin	
	175					180					185					
agc	aga	gtg	ata	tgc	ggt	gct	cac	tgg	caa	agc	gat	gtt	gat	gct	ggc	746
Ser	Arg	Val	He	(ys	Gly	Ala	His	Trp	Gln	Ser	Asp	Val	Asp	Ala	Gly	
190					195					200					205	
cgt	tat	gtg	gga	gca	gta	gag	ttt	gca	aga	ctg	caa	aca	atc	ccg	gct	794
Arg	Tyr	Val	Gly	Ala	Val	Glu	Phe	Ala	Arg	Leu	Gin	Thr	He	Pro	Ala	
				210					215					220		
ttt	cag	aag	tca	ctg	gca	aaa	tcc	gtg	agg	agc	tgaa	acga	aa a	aaata	aattta	847
		Lys														
			225					230								
ttga	igtaa	aag a	aagat	caco	c ca	aact	ttaat	t ta	ctgaa	aggt	gaaa	agtc	ttc	ccgca	aaactg	907
-		_	_						-			_		_	tgcctt	
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[0071]

<;210>; 6 <;211>; 232 <;212>; PRT

<:213>; Salmonella typhimurium

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<;400>; 6
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Tyr Thr Ser Ala Glu Thr Val Gln Pro Phe His Ser Pro Glu Glu Ser
             20
                                 25
Val Asn Ser Gln Phe Tyr Leu Pro Pro Pro Pro Gly Asn Asp Asp Pro
                             40
Ala Tyr Arg Tyr Asp Lys Glu Ala Tyr Phe Lys Gly Tyr Ala lle Lys
Gly Ser Pro Arg Trp Lys Gln Ala Ala Glu Asp Ala Asp Val Ser Val
                                         75
Glu Asn Ile Ala Arg Ile Phe Ser Pro Val Val Gly Ala Lys Ile Asn
                                     90
Pro Lys Asp Thr Pro Glu Thr Trp Asn Met Leu Lys Asn Leu Leu Thr
                                105
Met Gly Gly Tyr Tyr Ala Thr Ala Ser Ala Lys Lys Tyr Tyr Met Arg
                            120
Thr Arg Pro Phe Val Leu Phe Asn His Ser Thr Cys Arg Pro Glu Asp
                        135
                                            140
Glu Asn Thr Leu Arg Lys Asn Gly Ser Tyr Pro Ser Gly His Thr Ala
145
                                        155
Tyr Gly Thr Leu Leu Ala Leu Val Leu Ser Glu Ala Arg Pro Glu Arg
                                    170
Ala Gln Glu Leu Ala Arg Arg Gly Trp Glu Phe Gly Gln Ser Arg Val
lle Cys Gly Ala His Trp Gln Ser Asp Val Asp Ala Gly Arg Tyr Val
                            200
Gly Ala Val Glu Phe Ala Arg Leu Gln Thr lle Pro Ala Phe Gln Lys
                                            220
Ser Leu Ala Lys Ser Val Arg Ser
225
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acgccggaag gcttcatggt cgtcaaagtc gaaaagggta aagtcattcc gcattacgaa 120
agctatggct tccacacgat agacccgcgc aacacataat tgtcttatta tagccacatg 180
atatttttat attacaattt taaactaaaa ttaagaatta aattcttgaa ataaaqqttt 240
ttttattaaa aggataggaa atgtcgtgaa atcggcattt tctatccata ttatataaca 300
agggaagact gacgac atg ata aaa gtc ccg cgg ttc atc tgt atg atc gcg 352
                 Met lle Lys Val Pro Arg Phe lle Cys Met lle Ala
ctt aca tcc ggc gtt ctg gca agc ggc ctt tct caa agc gtt tca gct
                                                                  400
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Leu Thr Ser Gly Val Leu Ala Ser Gly Leu Ser Gln Ser Val Ser Ala

[0072]

		15					20					25				
cat	aca	gaa	aaa	agt	gaa	ссс	tcc	tcg	act	tat	cat	ttc	cac	agc	gat	448
His	Thr	Glu	Lys	Ser	Glu	Pro	Ser	Ser	Thr	Tyr	His	Phe	His	Ser	Asp	
	30					35					40					
ccc	ctt	ctt	tac	ctt	gcg	ccc	cca	ccc	act	tcc	ggc	agt	cca	tta	cag	496
Pro	Leu	Leu	Tyr	Leu	Ala	Pro	Pro	Pro	Thr	Ser	Gly	Ser	Pro	Leu	GIn	
45					50					55					60	
gcg	cat	gat	gat	caa	acc	ttt	aac	agc	acc	aga	caa	tta	aaa	ggt	agc	544
Ala	His	Asp	Asp	Gln	Thr	Phe	Asn	Ser	Thr	Arg	Gln	Leu	Lys	Gly	Ser	
				65					70					75		
acg	cgc	tgg	gca	ttg	gca	act	caa	gat	gcc	gat	ctt	cat	ctc	gct	tca	592
Thr	Arg	Trp	Ala	Leu	Ala	Thr	GIn	Asp	Ala	Asp	Leu	His	Leu	Ala	Ser	
			80					85					90			
gtt	ctc	aaa	gac	tat	gcc	tgc	gcc	gca	gga	atg	aat	ctc	gat	att	gcg	640
Val	Leu	Lys	Asp	Tyr	Ala	Cys	Ala	Ala	Gly	Met	Asn	Leu	Asp	He	Ala	
		95					100					105				
caa	tta	ccg	cat	ctt	gcc	aat	ttg	att	aaa	cgc	gca	ctt	cgc	acc	gaa	688
Gln	Leu	Pro	His	Leu	Ala	Asn	Leu	He	Lys	Arg	Ala	Leu	Arg	Thr	Glu	
	110					115					120					
tat	gac	gat	att	ggc	aga	gcc	aaa	aat	aac	tgg	aat	cgc	aaa	cga	cct	736
Tyr	Asp	Asp	He	Gly	Arg	Ala	Lys	Asn	Asn	Trp	Asn	Arg	Lys	Arg	Pro	
125					130					135					140	
ttt	gtg	gat	acc	gat	caa	ccc	atc	tgc	acg	gaa	aaa	gat	cgc	gaa	ggt	784
Phe	Val	Asp	Thr	Asp	GIn	Pro	He	Cys	Thr	Glu	Lys	Asp	Arg	Glu	Gly	
				145					150					155		
_			caa								_					832
Leu	Gly	Lys	Gln	Gly	Ser	Tyr	Pro	Ser	Gly	His	Thr	Thr	He	Gly	Trp	
			160					165					170			
_		_	ctc		_	_	-	_			_					880
Ser	Val		Leu	He	Leu	Ala	Glu	Leu	He	Pro	Asp	His	Ala	Ala	Asn	
		175					180					185				
			cgt													928
He		GIn	Arg	Gly	Gln			Gly	Thr	Ser		He	Val	Cys	Gly	
	190					195					200					
			ttc									_	-	_		976
	His	Trp	Phe	Ser	-	Val	GIn	Ala	Gly	-	He	Met	Ala	Ser	-	
205					210					215					220	
			gct						-		-	-	-	-	-	1024
Glu	He	Ala	Ala		His	Gly	Asp	Ala		Phe	Arg	Arg	Asp		Glu	
				225					230					235		
			aaa	_		_	-	-	-					-		1072
Leu	Ala	Arg	Lys	Glu	Leu	Glu	Lys		Arg	lhr	Ser	Ala		lhr	Pro	
			240	_				245					250			
-			cta	_	_		_		_	_	-	taa	attc	aat		1118
Asp	Asp		Leu	Cys	Lys	He		Gin	Ser	Ala	Arg					
	.4 4 -	255					260									4470
								-	-	_					acaggc	
-		-		_	_				-	_		_			cattct	
							-			_	cca	gata	act (gcaa	aattat	
agaa	ataco	cga o	cagct	tggaa	at a	tcgt	cact	t tt	ccta	g						1335

[0073] <;210>; 8 <;211>; 264 <;212>; PRT <;213>; Zymomonas mobilis <;400>; 8 Met lle Lys Val Pro Arg Phe lle Cys Met lle Ala Leu Thr Ser Gly Val Leu Ala Ser Gly Leu Ser Gln Ser Val Ser Ala His Thr Glu Lys 25 Ser Glu Pro Ser Ser Thr Tyr His Phe His Ser Asp Pro Leu Leu Tyr 40 Leu Ala Pro Pro Pro Thr Ser Gly Ser Pro Leu Gln Ala His Asp Asp GIn Thr Phe Asn Ser Thr Arg GIn Leu Lys Gly Ser Thr Arg Trp Ala 75 70 Leu Ala Thr Gln Asp Ala Asp Leu His Leu Ala Ser Val Leu Lys Asp 85 90 Tyr Ala Cys Ala Ala Gly Met Asn Leu Asp Ile Ala Gln Leu Pro His 105 Leu Ala Asn Leu Ile Lys Arg Ala Leu Arg Thr Glu Tyr Asp Asp Ile 120 Gly Arg Ala Lys Asn Asn Trp Asn Arg Lys Arg Pro Phe Val Asp Thr Asp Gin Pro Ile Cys Thr Glu Lys Asp Arg Glu Gly Leu Gly Lys Gln 155 Gly Ser Tyr Pro Ser Gly His Thr Thr Ile Gly Trp Ser Val Ala Leu lle Leu Ala Glu Leu Ile Pro Asp His Ala Ala Asn Ile Leu Gln Arg 185 Gly Gln Ile Phe Gly Thr Ser Arg Ile Val Cys Gly Ala His Trp Phe Ser Asp Val Gln Ala Gly Tyr lle Met Ala Ser Gly Glu lle Ala Ala 215 Leu His Gly Asp Ala Asp Phe Arg Arg Asp Met Glu Leu Ala Arg Lys 235 Glu Leu Glu Lys Ala Arg Thr Ser Ala His Thr Pro Asp Asp Leu Leu 245 250 255 Cys Lys Ile Glu Gln Ser Ala Arg 260 [0074] <;210>; 9 <;211>; 1650 <;212>; DNA <;213>; Enterobacter aerogenes <;220>; <;221>; CDS <;222>; (344).. (1087)

<;220>;

<;221>; mat#peptide

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Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val 180 185 gat gcg gcg cgg ata gtc ggc tcg gcg gtg gtg gcg acc ctg cat acc 1027 Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala Thr Leu His Thr 195 aac ccg gcc ttc caa cag cag ttg cag aaa gca aag gat gaa ttc gcc 1075 Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys Asp Glu Phe Ala 210 215 220 aaa acg cag aag taacgtcatc gccgttgaac tcccggaggc ggcgcttaac 1127 Lys Thr Gln Lys 225 gcgccttctc cgggctacta aatcgcacag cgctgtagcc ccggtaagcg ccagcgccac 1187 cggggatttt gagatagcca gcaccagtag tttcagccag cgtgatgaat acattaacgg 1247 caggccgcat gagtcgtaga tactgttatc ggtttgcaac ttttttaagg ttttttcccg 1307 gaggcggcgc gctgcgcctt ctccgggcta ctaaatcgca cagcgctgta gccccggtaa 1367 gcggcagcgc caccgggggt aacaagcgca gattcagaag cgcqtqacqa acqqcqcqqt 1427 atccgggcgc gtaaacatgg ttgatgcttt taactgcggc gtgccaaggt agaggaaacc 1487 gacaattttg tcctgttcgc ggcagccaaa gccttcgcgg acaaccggac tctcggttaa 1547 cgcaccgata cgccagatac cgttatagcc ctgcgccact gcggccattt gcatcgccat 1607 caccgcacat cccgcggaca tctcctgttc ccacagcggt acc 1650 <;210>; 10 <;211>; 248 <;212>; PRT <;213>; Enterobacter aerogenes <;400>; 10 Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro -1 Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys GIn Ala Gin Ala Asp Ala Asp Leu Ala Ala Gly Asp Val Ala Asn Ala Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln 85 Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala 100 Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe 115 120 Tyr Gly Val Ser Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys 130 135 140 Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Thr Gly Trp Ala Thr Ala 145 150 Leu Val Leu Ala Glu IIe Asn Pro Gln Arg Gln Asn Glu IIe Leu Lys

165

170

160

[0075]

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Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
                 GIn Ser Asp Val Asp Ala Ala Arg IIe Val Gly Ser Ala Val Val Ala
                 Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
                                     210
                                                         215
                 Asp Glu Phe Ala Lys Thr Gln Lys
                                 225
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                                   5
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<;211>; 25

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[0095]
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                        acid sequence around mutation
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                 <;211>; 25
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                 <;223>; Description of Artificial Sequence:primer
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[0100]
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                 <;211>; 25
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                  <;211>; 25
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                  <;211>; 7
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                        acid sequence around mutation
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                 <;223>; Description of Artificial Sequence:primer
                 <;400>; 41
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                 <;212>; DNA
                 <;213>; Artificial Sequence
                 <;220>;
                 <;223>; Description of Artificial Sequence:primer
                 <;400>; 42
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[0108]
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                 <;211>; 7
                 <;212>; PRT
                 <;213>; Artificial Sequence
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                 <;223>; Description of Artificial Sequence: partial amino
                       acid sequence around mutation
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                 <;213>; Artificial Sequence
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                 <;223>; Description of Artificial Sequence:primer
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                        acid sequence around mutation
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                                    5
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                  <;211>; 25
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                  <;213>; Artificial Sequence
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                  <;223>; Description of Artificial Sequence:primer
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                                                                                    25
[0113]
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                  <;211>; 25
                  <;212>; DNA
                  <;213>; Artificial Sequence
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                  <;223>; Description of Artificial Sequence:primer
                 <;400>; 48
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[0116]
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                                   5
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                       acid sequence around mutation
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                        acid sequence around mutation
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                                   5
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                                                                                   25
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                   1
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                                                                                   25
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                                                                                    25
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                                                                                    25
[0144]
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                   1
                                   5
[0145]
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[0146]
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                       acid sequence around mutation
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[0148]
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[0149]
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                                                                                   25
[0150]
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                       acid sequence around mutation
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                   1
                                   5
[0151]
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                        acid sequence around mutation
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[0154]
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                                                                                    25
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                                                                                    25
[0156]
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                 <;220>;
                 <;223>; Description of Artificial Sequence: partial amino
                       acid sequence around mutation
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Gln Asp Lys Phe Ser Lys Asn
[0157]
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                                                                                   25
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[0158]
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                 <;211>; 25
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                 <:223>; Description of Artificial Sequence:primer
                 <;400>; 93
                                                                                   25
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[0159]
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                 <;211>; 7
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                 <;213>; Artificial Sequence
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                 <;223>; Description of Artificial Sequence: partial amino
                       acid sequence around mutation
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                 GIn Asp Lys Lys Ser Lys Asn
                   1
                                    5
[0160]
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                 <;211>; 25
                 <;212>; DNA
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                                                                                   25
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[0161]
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                 <;211>; 25
                 <;212>; DNA
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                 <;223>; Description of Artificial Sequence:primer
                 <:400>; 96
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                        acid sequence around mutation
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[0163]
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                                                                                    30
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[0166]
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[0167]
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                 MUT300
[0168]
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                 <:223>; Description of Artificial Sequence:primer
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                                                                                   33
[0169]
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                 <;212>; DNA
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                                                                                   33
[0170]
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                 <;211>; 33
                 <;212>; DNA
                 <;213>; Artificial Sequence
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                                                                                   33
[0171]
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                 <;211>; 33
                 <;212>; DNA
                 <;213>; Artificial Sequence
                 <;220>;
                 <;223>; Description of Artificial Sequence:primer
                 <;400>; 106
                                                                                   33
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[0172]
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                 <;211>; 33
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<;220>;

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	<;211>; 33	
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	<;211>; 33	
	<;212>; DNA	
	<;213>; Artificial Sequence	
	<;220>;	
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[0175]		
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	<;211>; 33	
	<;212>; DNA	
	<;213>; Artificial Sequence	
	<;220>;	
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[0176]	3 3	33
	<;210>; 111	
	<;211>; 33	
	<;212>; DNA	
	<;213>; Artificial Sequence	
	721377 Merricial Sequence	
	<;220>;	
	<pre><:223>; Description of Artificial Sequence:primer</pre>	
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[0177]	<;210>; 112	
	<:211>; 33	
	<;212>; DNA	
	<pre><;213>; Artificial Sequence <;220>;</pre>	
	<pre><;223>; Description of Artificial Sequence:primer</pre>	
	<;400>; 112	
[0178]	cagatcgccg gcatcctga atcatatttgt cag	33
ro 1 2 0 1		

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                  <;211>; 33
                  <;212>; DNA
                  <;213>; Artificial Sequence
                  <;220>;
                  <:223>: Description of Artificial Sequence:primer
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[0179]
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                 <;212>; DNA
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                 <;220>;
                 <:223>; Description of Artificial Sequence:primer
                  <;400>; 114
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                                                                                   33
[0180]
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                 <;211>; 33
                 <;212>; DNA
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                 <;220>;
                 <:223>: Description of Artificial Sequence:primer
                 <;400>; 115
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                                                                                    33
[0181]
                 <;210>; 116
                 <;211>; 33
                 <;212>; DNA
                 <;213>; Artificial Sequence
                 <;220>;
                 <;223>; Description of Artificial Sequence:primer
                 <;400>; 116
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                                                                                   33
[0182]
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                 <;211>; 33
                 <;212>; DNA
                 <;213>; Artificial Sequence
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                 <;223>; Description of Artificial Sequence:primer
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                                                                                   33
[0183]
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                 <;211>; 33
                 <;212>; DNA
                 <;213>; Artificial Sequence
                 <;220>;
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[0184]
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                  <;211>; 33
                  <;212>; DNA
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                  <;400>; 119
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                                                                                    33
[0185]
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                  <;211>; 33
                  <;212>; DNA
                  <;213>; Artificial Sequence
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                  <;223>; Description of Artificial Sequence:primer
                  <;400>; 120
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                                                                                    33
[0186]
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                  <;211>; 9
                  <;212>; PRT
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                  <;220>;
                 <;223>; Description of Artificial Sequence: common motif
                        among acidic phosphatase family
                  <;220>;
                  <;221>; UNSURE
                  <;222>; (2, 3, 4, 5, 6, 7)
                  <;223>; X=optional amino acid
                  <;400>; 121
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                   1
[0187]
                  <;210>; 122
                  <;211>; 4
                  <;212>; PRT
                  <;213>; Artificial Sequence
                  <;220>;
                  <;223>; Description of Artificial Sequence: common motif
                       among acidic phosphatase family
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                 Pro Ser Gly His
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[0188]
                 <;210>; 123
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10

<;211>; 12 <;212>; PRT

<;213>; Artificial Sequence

<;220>;

<:223>: Description of Artificial Sequence: common motif
 among acidic phosphatase family

<;220>;

<;221>; UNSURE

<;222>; (3, 4, 5, 6, 7, 9, 10, 11)

<;223>; X=optional amino acid

<;400>; 123

Ser Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp

5

【図面の簡単な説明】

【図1】ホスファターゼ活性あるいはリン酸基転移活性を有する活性部位の構成要素となる5つのアミノ酸残基と、これらの空間的位置関係を $C\alpha$ 原子間の距離として示した図である。

【図2】EB-APのアミノ酸配列を、モルガネラ・モルガニ、サルモネラ・チフィムリウム、ザイモモナス・モビリス由来酸性ホスファターゼのアミノ酸配列とアラインメントした図である。

【図3】 E B - A P 反応中間体アナログとイノシンの結合様式モデルの結晶構造を示すコンピューターグラフィックス(CG)の写真である。

【図4】EB-APの6量体分子の結晶構造を示すCGの写真である。

【図5】EB-APのサブユニットの結晶構造を示すC Gの写真である。

【図6】EB-APの活性部位構造を示す図である。

【図7】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図8】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図9】EB-APとエンテロバクター・アエロゲネス 由来酸性ホスファターゼ(EA-AP)のアミノ酸配列 アラインメントをプログラムBLASTにより行った結果を 示す図である。

【図10】EB-APの構造の結晶学データ(1)を示す図である。

【図11】EB-APの構造の結晶学データ(2)を示す図である。

【図12】EB-APの構造の結晶学データ(3)を示す図である。

【図13】EB-APの構造の結晶学データ(4)を示す図である。

【図14】EB-APの構造の結晶学データ(5)を示す図である。

【図15】EB-APの構造の結晶学データ(6)を示す図である。

【図 1 6】 E B - A P の構造の結晶学データ (7) を示す図である。

【図 1 7】 E B - A P の構造の結晶学データ (8) を示す図である。

【図18】 EB-APの構造の結晶学データ (9) を示す図である。

【図19】EB-APの構造の結晶学データ(10)を示す図である。

【図20】EB-APの構造の結晶学データ(11)を示す図である。

【図21】EB-APの構造の結晶学データ(12)を 示す図である。

【図22】EB-APの構造の結晶学データ(13)を示す図である。

【図23】 EB-APの構造の結晶学データ (14) を 示す図である。

【図24】 EB-APの構造の結晶学データ (15) を示す図である。

【図25】EB-APの構造の結晶学データ(16)を 示す図である。

【図26】EB-APの構造の結晶学データ(17)を 示す図である。

【図27】EB-APの構造の結晶学データ(18)を示す図である。

【図28】EB-APの構造の結晶学データ(19)を示す図である。

【図29】 E B - A P の構造の結晶学データ (20) を示す図である。

【図30】 EB-APの構造の結晶学データ (21) を示す図である。

【図31】 EB-APの構造の結晶学データ (22) を示す図である。

【図32】 E B - A P の構造の結晶学データ (23) を示す図である。

【図33】EB-APの構造の結晶学データ(24)を示す図である。

【図34】 EB-APの構造の結晶学データ(25)を

示す図である。

【図35】EB-APの構造の結晶学データ(26)を示す図である。

【図36】 E B – A P の構造の結晶学データ (27) を示す図である。

【図37】 EB-APの構造の結晶学データ (28) を示す図である。

【図38】 EB-APの構造の結晶学データ (29) を示す図である。

【図39】 E B - A P の構造の結晶学データ (30) を示す図である。

【図40】EB-APの構造の結晶学データ(31)を示す図である。

【図41】 EB-APの構造の結晶学データ (32) を示す図である。

【図42】 EB-APの構造の結晶学データ (33) を示す図である。

【図43】 EB-APの構造の結晶学データ (34) を示す図である。

【図44】 EB-APの構造の結晶学データ (35) を示す図である。

【図45】 EB-APの構造の結晶学データ (36) を示す図である。

【図46】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図47】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図48】部位特異的突然変異誘発法に使用したプライマーセットを示す図である。

【図1】

Arg(N末側)
His(C末側) 4.5-6.7

9.4-11.8

10.4-12.6

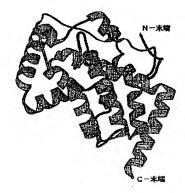
12.2-15.2

10.7-13.6

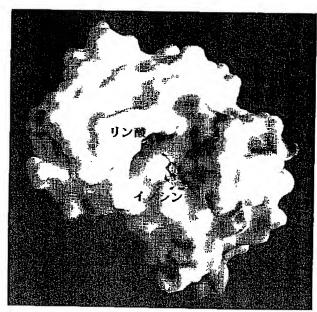
Lys

His(N末側)

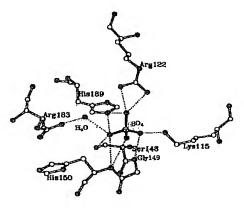
【図5】



【図3】



【図6】



210

【図2】 140 70 GKLAAEDANL KGYAILGSPR WKQAAEDADV GKOADADADL LAPPPTSGSP LQAHDDQTFN STRQLKGSTR WALATQDADL

g

22

g

KGRMLRNTER

OFLNDQAMYE

LPPPEVGSI

LPPPPAVGSI AFLNDOAMYE

NSEAINSLAL

TIKEDLYYLK

LALVATGNDT AIPAGNDA LPPPGNDDP AYRYDKEAYF

PERSVINSOFY

SAETVOPFES HTEKSEPSST TIKPDLYYLK

KYT

S. typhimurium

S.mobilis

M.morganii

E.blattae

:ASGLSQSVSA

YHPHSDPLLY

NEGAIDSLEL

拉特洛 李

OGRLLRNTER

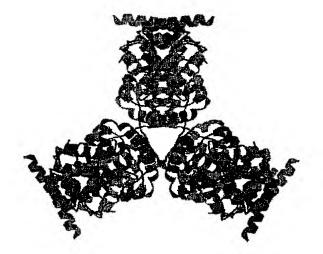
TCNTTEODKL TCRPEDENTL TCNTKDOKKL ICTEKDREGL TRPFAFYGVS TRPEVLFINE GR-AKNIWNR KRPFVDTDOP IRPPAFYGTE 80 TASAKKYYMR TRSAKEHYMR TRSAKDHYMR 8 DAPALHKLLT NMIEDAGDLA NMIEDAGDLA NLLTMGGYYA OLPHLANLIK RALRTEYDDI 0,7 DSPELYKLLT DIPETWINER HLASVLKDYA CAAGMILDIA SSGGVANAFS CAFGSPITER GAFGYPITER SVENIARIFS PVVGAKINPK . AAGGVATAFS Q S. typhimurium M.morganii E.blattae Z.mobilis

GENTICGYHW OSHVDAARIV GSAVVATLHT GERVICGYHW OSHVDAARIV GSAAVATLHS GSRVICGAHW OSHVDAGRYV GAVEFARIOT TSRIVCGAHW FSDVQAGYIM ASGEIAALHG lsearperag elarrgwefg Laelipdhaa nilorgoifg AILERGYOLG EILKRGYELG LAEINPORON LAEVNEANOD 如如 RKNGSYPSGH TAYGTLIALV GRQGSYPSGH TIGWSVALI *** TSIGWATALV STNGSYPSCH TSIGWATALV SKNGSYPSGH S. typhimurium M.morganii E. blattae .mobilis

 α 13

SAHTPDDLLC KIEGSAR LLSKEDHPKL DADFREDMEL ARKELERART : IPAPQKSLAK VREELNDKUN DPAFQAQLAK AKQEFAQKSQ NPAFOQOLOK AKAEFAQHOK S. typhimurium M.morganii S.mobilis E.blattae

【図4】



【図7】

S72F(s) 5'-CA-AAC-CTG-AGC-TTT-GGC-GAT-GTG-GC-3' S72F(as) 3'-GT-TTG-GAC-TCG-AAA-CCG-CTA-CAC-CG-5' M L S F72 G D V S72Y(B) 5'-CA-AAC-CTG-AGC-TAC-GGC-GAT-GTG-GC-3' S72Y(as) 3'-GT-TTG-GAC-TCG-ATG-CCG-CTA-CAC-CG-5' K L S Y72 G D V 5'-CA-AAC-CTG-AGC-TGG-GGC-GAT-GTG-GC-3' S72W(s) S72W(as) 3'-GT-TTG-GAC-TCG-ACC-CCG-CTA-CAC-CG-5' L S W72 G D V H S72D(s) 5'-CA-AAC-CTG-AGC-GAC-GGC-GAT-GTG-GC-3' S72D(as) 3'-GT-TTG-GAC-TCG-CTG-CCG-CTA-CAC-CG-5' N L S D72 G D V S72V(8) 5'-CA-AAC-CTG-AGC-GTT-GGC-GAT-GTG-GC-3' S72V(as) 3'-GT-TTG-GAC-TCG-CAA-CCG-CTA-CAC-CG-5' N L S V72 G D V S72E(s) 5'-CA-AAC-CTG-AGC-GAA-GGC-GAT-GTG-GC-3' S72E(as) 3'-GT-TTG-GAC-TCG-CTT-CCG-CTA-CAC-CG-5' N L S E72 G D V S72H(s) 5'-CA-AAC-CTG-AGC-ATG-GGC-GAT-GTG-GC-3' S72M(as) 3'-GT-TTG-GAC-TCG-TAC-CCG-CTA-CAC-CG-5' N L S M72 G D 572T(B) 5'-CA-AAC-CTG-AGC-ACC-GGC-GAT-GTG-GC-3' S72T(as) 3'-GT-TTG-GAC-TCG-TGG-CCG-CTA-CAC-CG-5' N L S T72 G D V S72L(s) 5'-CA-AAC-CTG-AGC-CTG-GGC-GAT-GTG-GC-3' S72L(as) 3'-GT-TTG-GAC-TCG-GAC-CCG-CTA-CAC-CG-5' H L S L72 G D V S72R(s) 5'-CA-AAC-CTG-AGC-CGT-GGC-GAT-GTG-GC-3' S72R(as) 3'-GT-TTG-GAC-TCG-GCA-CCG-CTA-CAC-CG-5' N L S R72 G D V S72Q(s) 5'-CA-AAC-CTG-AGC-CAG-GGC-GAT-GTG-GC-3' S72Q(as) 3'-GT-TTG-GAC-TCG-GTC-CCG-CTA-CAC-CG-5' H L S Q72 G D V S72K(B) 5'-CA-AAC-CTG-AGC-AAA-GGC-GAT-GTG-GC-3' S72K(as) 3'-GT-TTG-GAC-TCG-TTT-CCG-CTA-CAC-CG-5' N L S K72 G D Y S72P(s) 5'-CA-AAC-CTG-AGC-CCG-GGC-GAT-GTG-GC-3' S72P(as) 3'-GT-TTG-GAC-TCG-GGC-CCG-CTA-CAC-CG-5' N L S P72 G D V S72A(s) 5'-CA-AAC-CTG-AGC-GCG-GGC-GAT-GTG-GC-3' S72A(as) 3'-GT-TTG-GAC-TCG-CGC-CCG-CTA-CAC-CG-5' L S A72 G D S72N(s) 5'-CA-AAC-CTG-AGC-AAC-GGC-GAT-GTG-GC-3' S72H(as) 3'-GT-TTG-GAC-TCG-TTG-CCG-CTA-CAC-CG-5' N L S N72 G D V 572G(s) 5'-CA-AAC-CTG-AGC-GGT-GGC-GAT-GTG-GC-3' S72G(as) 3'-GT-TTG-GAC-TCG-CCA-CCG-CTA-CAC-CG-5' N L S G72 G D V S72H(s) 5'-CA-AAC-CTG-AGC-CAC-GGC-GAT-GTG-GC-3' S72H(as) 3'-GT-TTG-GAC-TCG-GTG-CCG-CTA-CAC-CG-5' N L S H72 G D V

[図8]

L16W(S) 5'-CG-AAA-CCG-GAT-TGG-TAC-TAC-CTC-AA-3' L16W(as) 3'-GC-TTT-GGC-CTA-ACC-ATG-ATG-GAG-TT-5' K P D W16 Y Y S71W(s) 5'-AT-GCA-AAC-CTG-TGG-AGT-GGC-GAT-GT-3' S71W(as) 3'-TA-CGT-TTG-GAC-ACC-TCA-CCG-CTA-CA-5' A N L W71 S G D G73W(s) 5'-AC-CTG-AGC-AGT-TGG-GAT-GTG-GCG-AA-3' G73W(as) 3'-TG-GAC-TCG-TCA-ACC-CTA-CAC-CGC-TT-5' L S S W73 D V A E104F(s) 5'-CC-AAT-ATG-ATT-TTT-GAC-GCC-GGG-GA-3' E104F(as) 3'-GG-TTA-TAC-TAA-AAA-CTG-CGG-CCC-CT-5' M I F104 D A E104W(S) 5'-CC-AAT-ATG-ATT-TGG-GAC-GCC-GGG-GA-3' E104W(as) 3'-GG-TTA-TAC-TAA-ACC-CTG-CGG-CCC-CT-5' N M I W104 D A G

【図9】

EB-AP:	LALVATGNDTTTKPDLYYLKNSEAINSLALLPPPPAVGSIAFLNDQAMYEQGRLLRNTER V GND TTKPDLYYLKN++AI+SLALLPPPP VGSIAFLNDQAMYE+GRLLRNTER LVPAGNDATTKPDLYYLKNAQAIDSLALLPPPPEVGSIAFLNDQAMYEKGRLLRNTER
	[72]
EB-AP:	GKLAAEDANLSSGGVANAFSGAFGSPITEKDAPALHKLLTNMIEDAGDLATRSAKDHYMR
	GKLAAEDANLS+GGVANAFS AFGSPITEKDAP LHKLLTNMIEDAGDLATRSAK+ YMR
EA-AP:	GKLAAEDANLSAGGVANAFSSAFGSPITEKDAPQLHKLLTNMIEDAGDLATRSAKEKYMR [70]
EB-AP:	IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
	IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
EA-AP:	IRPFAFYGVSTCNTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELG
	TOWNER TO A TOWNER OF THE PROPERTY OF THE PROP
EB-AP:	QSRVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAKAEFAQHQKK
	+SRVICGYHWQSDVDAAR+VGSAVVATLHTNPAFQQQLQKAK EFA+ QK
EA-AP:	ESRVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKTQK

【図10】

ATOM	1	N	GLY	Α	7	35.965	71.208	89.712	1.00 36.	57
ATOM	2	CA	GLY	A	7	37.459	71.295	89.574	1.00 31.	92
ATOM	3	С	GLY	A	7	38.160	69.982	89.872	1.00 29.	
MOTA	4	0	GLY		7	39.301	69.858	89.492	1.00 31.	81
ATOM	5	N	ASN		8	37.485	68.990	90.532	1.00 26.	40
ATOM	6	CA	ASN	A	8	38.284	67.775	90.697	1.00 26.	63
ATOM	7	С	ASN	A	8	38.466	67.018	89.396	1.00 29.	21
MOTA	8	0	ASN	Α	В	37.736	67.238	88.431	1.00 30.	52
ATOM	9	CB	ASN	A	8	37.677	66.810	91.702	1.00 27.	01
ATOM	10	CG	ASN	A	В	37.725	67.396	93.104	1.00 32.	45
ATOM	11	OD1	ASN	A	8	38.751	67.744	93.636	1.00 30.	02
ATOM	12	ND2	ASN	A	8	36.545	67.536	93.707	1.00 31.	60
MOTA	13	N	ASP	A	9	39.455	66.154	89.463	1.00 29.	14
MOTA	14	CA	ASP	A	9	39.787	65.216	88.391	1.00 30.	47
ATOM	15	C	ASP	A	9	40.661	64.081	88.901	1.00 31.	02
MOTA	16	0	ASP	A	9	40.804	63.931	90.110	1.00 31.	00
MOTA	17	CB	ASP	A	9	40.394	65.960	87.195	1.00 30.	92
ATOM	18	CG	ASP	A	9	41.802	66.484	87.429	1.00 32.	66
ATOM	19	OD1	ASP	A	9	42.307	66.333	88.532	1.00 35.	03
ATOM	20	OD2	ASP	A	9	42.400	67.018	86.493	1.00 31.	63
ATOM	21	N	THR	A	10	41.272	63.298	87.998	1.00 28.	72
ATOM	22	CA	THR	A	10	42.188	62.228	88.430	1.00 28.	53
ATOM	23	С	THR	A	10	43.408	62.655	89.259	1.00 30.3	10
MOTA	24	0	THR	A	10	43.946	61.944	90.095	1.00 29.0	06
ATOM	25	CB	THR	A	10	42.692	61.405	87.235	1.00 26.0	05
ATOM	26	OG1	THR	A	10	43.272	60.172	87.655	1.00 27.	75
MOTA	27	CG2	THR	A	10	43.670	62.174	86.313	1.00 23.	76
ATOM	28	N	THR	A	11	43.814	63.900	88.996	1.00 30.4	82
ATOM	29	CA	THR	A	11	44.932	64.389	89.799	1.00 32.	79
ATOM	30	С	THR	A	11	44.605	64.736	91.267	1.00 36.3	32
ATOM	31	0	THR	A	11	45.435	64.658	92.162	1.00 37.2	21
MOTA	32	CB	THR	A	11	45.588	65.591	89.143	1.00 30.5	53
ATOM	33	OG1	THR	A	11	44.845	66.781	89.359	1.00 27.3	79
ATOM	34	CG2	THR	A	11	45.899	65.362	87.656	1.00 32.1	16
ATOM	35	N	THR	A	12	43.317	65.076	91.495	1.00 34.8	31
ATOM	36	CA	THR	A	12	42.910	65.213	92.900	1.00 32.9	91
ATOM	37	C	THR	A	12	42.265	63.992	93.549	1.00 33.0	80
ATOM	38	0	THR	A	12	42.350	63.742	94.736	1.00 32.4	19
ATOM	39	CB	THR	A	12	41.963	66.395	93.077	1.00 30.9	92
ATOM	40	OG1	THR	A	12	40.719	66.162	92.409	1.00 32.0) 4
ATOM	41	CG2	THR		12	42.599	67.667	92.543	1.00 29.7	75
ATOM	42	N	LYS	A	13	41.565	63.229	92.703	1.00 31.1	.7
ATOM	43	CA	LYS		13	40.791	62.064	93.174	1.00 30.2	27
ATOM	44	С	LYS		13	40.904	60.812	92.287	1.00.31.4	10
MOTA	45	0	LYS		13	39.981	60.348	91.605	1.00 33.0	15
ATOM	46	CB	LYS	A	13	39.294	62.395	93.331	1.00 29.0	9
ATOM	47	CG	LYS		13	39.001	63.747	93.965	1.00 32.9	
ATOM	48	CD	LYS		1.3	37.536	64.076	94.166	1.00 37.8	
ATOM	49	CE	LYS		13	36.767	62.909	94.772	1.00 47.2	
ATOM	50	NZ	LYS		13	35.340	63.270	94.947	1.00 52.0	
ATOM	51	N	PRO		14	42.138	60.283	92.279	1.00 33.0)1
ATOM	52	CA	PRO		14	42.516	59.249	91.290	1.00 32.0	6
ATOM	53	C	PRO		14	41.823	57.907	91.452	1.00 30.9	8
ATOM	54	0	PRO	A	14	41.961	56.989	90.668	1.00 32.5	7

【図11】

ATOM	55	CB	PRO	A	14	44.035	59.145	91.468	1.00	34.46
ATOM	56	CG	PRO	A	14	44.283	59.564	92.920	1.00	33.02
ATOM	57	CD	PRO	A	14	43.225	60.638	93.181	1.00	34.46
ATOM	58	M	ASP	Α	15	41.046	57.815	92.513	1.00	29.27
MOTA	59	CA	ASP	A	15	40.204	56.655	92.809	1.00	28.89
MOTA	60	С	ASP	A	15	38.810	56.684	92,146	1.00	21.76
ATOM	61	0	ASP		15	38.078	55.706	92.030	1.00	20.59
ATOM	62	CB	ASP	A	15	40.125	56.599	94.368	1.00	37.60
ATOM	63	CG	ASP	A	15	39.589	57.903	95.080		45.11
ATOM	64	OD1			15	40.062	59.044	94.817		45.67
ATOM	65	OD2			15	38.687	57.751	95.922	1.00	
ATOM	66	N	LEU		16	38.495	57.910	91.726		20.49
MOTA	67	CA	LEU		16	37.182	58.179	91.135	1.00	
ATOM	68	С	LEU		16	37.156	58.814	89.727	1.00	
ATOM	69	ō	LEU		16	36.109	59.011	89.134	1.00	
ATOM	70	СВ	LEU		16	36.354	59.099	92.029	1.00	
ATOM	71	CG	LEU		16	35.814	58.432	93.297		25.48
ATOM	72	CD1			16	34.876	57.253	93.075		24.05
ATOM	73	CD2			16	35.092	59.477	94.104		25.22
ATOM	74	N	TYR		17	38.343	59.175	89.273		20.96
ATOM	75	CA	TYR		17		59.605			
ATOM	76	C	TYR		17	38.555 39.780		87.889		22.04
ATOM	77	o					58.903	87.334		22.80
ATOM	78	СВ	TYR		17	40.790	58.799	88.021		23.48
			TYR		17	38.856	61.095	87.711		18.01
ATOM	79	CG	TYR		17	37.928	62.099	88.371		24.78
ATOM	80	CD1	TYR		17	37.129	62.916	87.542		22.78
ATOM	81	CD2	TYR		17	37.905	62.248	89.781		23.58
ATOM	82	CE1	TYR		17	36.317	63.919	88.113		26.51
ATOM	83	CE2	TYR		17	37.090	63.240	90.349		22.88
ATOM	84	CZ	TYR		17	36.303	64.059	89.517		24.63
ATOM	85	OH	TYR		17	35.482	65.023	90.066		22.92
ATOM	86	N	TYR		18	39.670	58.482	86.053		26.17
ATOM	87	CA	TYR		18	40.838	58.209	85.191		21.13
ATOM	88	С	TYR		18	41.332	59.414	84.464	1.00	19.92
ATOM	89	0	TYR	A	18	42.490	59.511	84.083	1.00	22.64
ATOM	90	CB	TYR	A	18	40.563	57.195	84.080	1.00	17.53
ATOM	91	CG	TYR		18	40.312	55.826	84.610	1.00	16.91
atom	92	CD1	TYR	A	18	41.425	55.028	84.916	1.00	19.86
MOTA	93	CD2	TYR	A	18	38.985	55.372	84.771	1.00	16.65
ATOM	94	CE1	TYR	A	18	41.218	53.725	85.383	1.00	18.64
ATOM	95	CE2	TYR	A	18	38.765	54.053	85,213	1.00	17.52
ATOM	96	CZ	TYR	A	18	39.892	53.262	85.515	1.00	21.18
MOTA	97	OH	TYR	A	18	39.734	51.974	85.977	1.00	26.15
MOTA	98	N	LEU	A	19	40.412	60.336	84.236	1.00	21.49
ATOM	99	CA	LEU	A	19	40.788	61.462	83.366		22.71
MOTA	100	C	LEU	A	19	41.094	62.812	84.021	1.00	25.01
ATOM	101	0	LEU	A	19	40.771	63.125	85.159		25.24
ATOM	102	CB	LEU		19	39.708	61.669	82.290		21.68
ATOM	103	CG	LEU		19	39.301	60.442	81.432		22.88
ATOM	104		LEU		19	40.430	59.842	80.583		20.39
ATOM	105		LEU		19	38.078	60.812	80.608		18.83
ATOM	106	N	LYS		20	41.736	63.667	83.246		26.44
ATOM	107	CA	LYS		20	41.947	65.032	83.717		26.77
ATOM	108	C	LYS		20	40.935	66.034	83.292		
		~		44	~~	×0.333	00.034	03.494	T.00	26.42

【図12】

ATOM	109	0	LYS	A	20	40.182	65.870	82.341	1.00 29.05
ATOM	110	CB	LYS	Α	20	43.239	65.608	83.187	1.00 30.61
ATOM	111	CG	LYS	A	20	44.400	64.791	83.648	1.00 32.90
ATOM	112	CD	LYS	A	20	45.633	65.326	82.963	1.00 39.72
ATOM	113	CE	LYS	A	20	46.698	64.259	83.113	1.00 50.27
ATOM	114	NZ	LYS		20	46.148	62.977	82.610	1.00 62.00
ATOM	115	N	ASN		21	41.050	67.184	83.943	1.00 24.09
ATOM	116	CA	ASN		21	40.154	68.246	83.530	1.00 23.98
ATOM	117	C	ASN		21	40.177	68.539	82.032	1.00 25.08
ATOM	118	ŏ	ASN		21	39.134	68.722	81.427	1.00 25.36
ATOM	119	СВ	ASN		21	40.310	69.512	84.371	1.00 23.81
ATOM	120	CG	ASN		21	39.601	69.311		
ATOM	121	OD1			21			85.697	1.00 26.97
						38.392	69.175	85.836	1.00 26.36
ATOM	122	ND2			21	40.403	69.303	86.744	1.00 32.00
MOTA	123	N	SER		22	41.378	68.486	81.450	1.00 23.72
MOTA	124	CA	SER		22	41.592	68.804	80.008	1.00 25.53
ATOM	125	C	SER		22	40.992	67.752	79.068	1.00 25.77
ATOM	126	0	SER		22	40.524	68.007	77.966	1.00 27.65
ATOM	127	CB	SER	A	22	43.079	68.868	79.699	1.00 23.34
ATOM	128	OG	SER	A	22	43.719	67.716	80.303	1.00 33.30
ATOM	129	N	GLU	A	23	40.957	66.529	79.624	1.00 22.35
ATOM	130	CA	GLU	A	23	40.320	65.466	78.899	1.00 21.87
ATOM	131	С	GLU	A	23	38.811	65.375	78.974	1.00 23.18
ATOM	132	0	GLU	A	23	38.197	64.451	78.471	1.00 25.83
ATOM	133	CB	GLU	A	23	40.923	64.165	79.337	1.00 22.13
ATOM	134	CG	GLU		23	42.451	64.215	79.214	1.00 26.78
ATOM	135	CD	GLU		23	43.021	62.908	79.718	1.00 30.40
ATOM	136	OE1	GLU		23	42.946	62.648	80.900	1.00 31.10
MOTA	137	OE2	GLU		23	43.544	62.118	78.957	
ATOM	138	N	ALA		24				
ATOM	139	CA				38.196	66.359	79.610	1.00 21.49
			ALA		24	36.751	66.165	79.738	1.00 22.48
ATOM	140	C	ALA		24	35.973	66.420	78.438	1.00 22.81
ATOM	141	0	ALA		24	36.325	67.333	77.704	1.00 23.36
ATOM	142	CB	ALA		24	36.188	67.183	80.734	1.00 21.43
ATOM	143	N	ILE		25	34.859	65.694	78.228	1.00 23.46
ATOM	144	CA	ILE		25	33.845	66.149	77.243	1.00 23.60
ATOM	145	¢	ILE		25	33.312	67.535	77.530	1.00 24.71
ATOM	146	0	ILE	A	25	32.788	67.809	78.603	1.00 25.03
ATOM	147	CB	ILE	A	25	32.684	65.160	77.096	1.00 20.26
ATOM	148	CG1	ILE	A	25	33.237	63.749	76.838	1.00 23.14
ATOM	149	CG2	ILE	A	25	31.739	65.555	75.954	1.00 21.26
ATOM	150	CD1	ILE	A	25	34.298	63.551	75.722	1.00 16.94
ATOM	151	N	ASN	A	26	33.485	68.431	76.562	1.00 22.50
ATOM	152	CA	ASN		26	32.797	69.706	76.751	1.00 22.04
ATOM	153	С	ASN		26	31.295	69.680	76.533	1.00 22.52
ATOM	154	ō	ASN		26	30.731	70.042	75.509	1.00 22.34
ATOM	155	СВ	ASN		26	33.474	70.744		1.00 20.15
ATOM	156	CG	ASN		26	32.982	72.133	75.900 76.217	
ATOM	157		ASN			31.923			1.00 24.94
ATOM	158		ASN		26		72.459	76.732	1.00 29.46
ATOM	159				26	33.827	73.032	75.809	1.00 25.76
		N	SER		27	30.627	69.289	77.622	1.00 19.77
ATOM	160	CA	SER		27	29.166	69.168	77.549	1.00 18.88
ATOM	161	C	SER		27	28.412	70.423	77.177	1.00 18.74
ATOM	162	0	SER	A	27	27.390	70.393	76.517	1.00 21.73

[図13]

ATOM	163	CB	SER	A	27	28.606	68.619	78.870	1.00	19.35
ATOM	164	OG	SER	A	27	28.967	69.518	79.940	1.00	19.36
ATOM	165	N	LEU	A	28	28.961	71.564	77.588		18.08
ATOM	166	CA	LEU	A	28	28.271	72.815	77.262		20.15
ATOM	167	С	LEU	A	28	28.283	73.062	75.761		23.12
ATOM	168	0	LEU	A	28	27.303	73.485	75.165		22.25
ATOM	169	CB	LEU	Α	28	28.990	74.042	77.798		17.00
ATOM	170	CG	LEU	A	28	28.159	75.188	78.376		18.01
ATOM	171	CD1	LEU		28	26.847	75.547	77.733		14.28
MOTA	172	CD2			28	29.053	76.394	78.592		16.45
MOTA	173	N	ALA		29	29,478	72.767	75.193		23.87
ATOM	174	CA	ALA		29	29.598	72.827	73.707		22.62
ATOM	175	С	ALA		29	28.773	71.847	72.837		20.86
ATOM	176	o	ALA		29	28.192	72.239	71.830		25.89
ATOM	177	СВ	ALA		29	31.065	72.692	73.326		20.24
ATOM	178	N	LEU		30	28.733	70.580	73.267		15.78
ATOM	179	CA	LEU		30	28.079	69.497	72.519		18.05
ATOM	180	C	LEU		30	26.557	69.416	72.559		22.38
ATOM	181	ō	LEU		30	25.845	69.251	71.566		23.63
ATOM	182	СВ	LEU		30	28.732	68.194	72.977		16.47
ATOM	183	CG	LEU		30	28.234	66.887	72.360		17.59
ATOM	184	CDI	LEU		30	28.812	65.706	73.120	1.00	
ATOM	185	CD2	LEU		30	28.456	66.775	70.850		
ATOM	186	N	LEU		31	26.436		-		13.89
ATOM	187	CA	LEU		31	24.633	69.533	73.812		22.39
ATOM	188	C	LEU		31	23.817	69.430	74.049		18.84
ATOM	189	o	LEU		31		70.624	73.538		16.30
ATOM	190	CB	LEU			24.260	71.763	73.576		19.93
					31	24.381	69.199	75.556		16.94
ATOM	191	CG	LEU		31	24.923	67.873	76.095		17.95
ATOM	192	CD1	LEU		31	24.177	66.669	75.553		11.60
ATOM	193	CD2	LEU		31	24.823	67.878	77.628		18.77
ATOM	194	N	PRO		32	22.581	70.333	73.105		14.BO
ATOM	195	CA	PRO		32	21.589	71.404	72.910		18.31
ATOM	196	C	PRO		32	21.228	72.028	74.278		22.24
ATOM	197	0	PRO		32	21.453	71.442	75.327		22.27
ATOM	198	CB	PRO		32	20.402	70.621	72.348		15.94
ATOM	199	CG	PRO		32	20.545	69.184	72.847		16.93
ATOM	200	CD	PRO		32	22.038	68.972	72.954		15.56
ATOM	201	N	PRO		33	20.657	73.249	74.287	1.00	23.41
ATOM	202	ÇA	PRO		33	20.190	73.780	75.586	1.00	20.34
ATOM	203	C	PRO		33	19.059	72.945	76.084	1.00	19.93
ATOM	204	0	PRO		33	18.409	72.292	75.285		18.67
ATOM	205	CB	PRO		33	19.659	75.158	75.224		18.52
ATOM	206	CG	PRO		33	20.267	75.499	73.877	1.00	21.71
ATOM	207	CD	PRO	A	33	20.406	74.146	73.177	1.00	21.12
MOTA	208	N	PRO	A	34	18.785	72.950	77.411	1.00	19.36
ATOM	209	CA	PRO	A	34	17.645	72.138	77.863	1.00	13.70
MOTA	210	С	PRO		34	16.348	72.759	77.351		11.77
ATOM	211	0	PRO		34	16.280	73.937	77.090		14.58
ATOM	212	CB	PRO	A	34	17.760	72.358	79.389		13.66
ATOM	213	CG	PRO	A	34	18.471	73.698	79.571	1.00	14.81
ATOM	214	CD	PRO	A	34	19.499	73.679	78.464	1.00	16.49
ATOM	215	N	PRO		35	15.257	72.007	77.284		12.52
MOTA	216	CA	PRO	A	35	14.011	72.710	76.973		13.71

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MOTA	217	C	PRO	A	35	13.665	73.842	77.945	1.00	20.26
MOTA	218	0	PRO	A	35	13.728	73.715	79.159	1.00	20.52
MOTA	219	CB	PRO	A	35	12.997	71.579	76.991	1.00	11.74
ATOM	220	CG	PRO	A	35	13.723	70.243	77.051	1.00	12.04
ATOM	221	CD	PRO	A	35	15.140	70.581	77.482		11.57
ATOM	222	N	ALA	A	36	13,311	74.962	77.356	1.00	19.25
ATOM	223	CA	ALA	A	36	12.919	76.136	78.122		18.78
ATOM	224	С	ALA	A	36	11.457	76.120	78.497	1.00	18.25
ATOM	225	0	ALA	A	36	10.582	75.579	77.847		18.88
ATOM	226	CB	ALA	A	36	13.152	77.414	77.304		17.95
ATOM	227	N	VAL	A	37	11.182	76.753	79.609		18.03
ATOM	228	CA	VAL	A	37	9.803	77.005	79.965		16.78
ATOM	229	C	VAL	A	37	9.135	77.993	78.998		17.18
ATOM	230	0	VAL	A	37	9.640	79.048	78.650	1.00	
ATOM	231	CB	VAL	A	37	9.740	77.500	81.436	1.00	
ATOM	232	CG1	VAL	A	37	10.381	76.501	82.418	1.00	
ATOM	233	CG2	VAL	A	37	8.300	77.825	81.832	1.00	
MOTA	234	N	GLY	A	38	7.952	77.616	78.561		18.34
ATOM	235	CA	GLY		38	7.422	78.249	77.343		22.06
ATOM	236	C	GLY	A	38	7.538	77.398	76.043		21.25
ATOM	237	0	GLY	A	38	6.851	77.623	75.068		22.09
ATOM	238	N	SER	A	39	8.422	76.401	76.060		21.73
ATOM	239	CA	SER	A	39	8.520	75.487	74.905		20.30
ATOM	240	С	SER	A	39	7.604	74.277	74.964		21.10
ATOM	241	0	SER	A	39	7.217	73.736	76.002		19.55
ATOM	242	СВ	SER	A	39	9.946	74.998	74.748		15.45
MOTA	243	OG	SER	A	39	10.197	73.967	75.704		15.38
ATOM	244	N	ILE	A	40	7.287	73.796	73.772		17.17
ATOM	245	CA	ILE	A	40	6.618	72.485	73.702		14.71
ATOM	246	C	ILE	A	40	7.475	71.311	74.225		10.81
ATOM	247	0	ILE	A	40	6.998	70.315	74.782		15.23
ATOM	248	CB	ILE	A	40	6.102	72.235	72.219		15.78
ATOM	249	CG1	ILE	A	40	5.162	73.368	71.791		15.41
ATOM	250	CG2	ILE	A	40	5.406	70.863	72.091		14.54
ATOM	251	CD1			40	4.812	73.332	70.307		18.26
MOTA	252	N	ALA	A	41	8.790	71.443	74.040		10.69
ATOM	253	CA	ALA	A	41	9.633	70.373	74.530	1.00	
ATOM	254	С	ALA	A	41	9.566	70.300	76.091		15.36
ATOM	255	0	ALA	A	41	9.369	69.245	76.683	1.00	
ATOM	256	CB	ALA	A	41	11.046	70.610	74.065	1.00	
ATOM	257	N	PHE	A	42	9.547	71.495	76.702	1.00	
ATOM	258	CA	PHE	A	42	9.200	71.480	78.151	1.00	
ATOM	259	С	PHE		42	7.818	70.970	78.533		16.07
ATOM	260	0	PHE	A	42	7.652	70.182	79.448		19.72
ATOM	261	СВ	PHE	A	42	9.513	72.819	78.819		17.93
ATOM	262	CG	PHE	A	42	9.380	72.700	80.338	1.00	
ATOM	263	CD1	PHE		42	10.297	71.904	81.056	1.00	
ATOM	264		PHE		42	8.324	73.370	80.997	1.00	
ATOM	265		PHE		42	10.148	71.763	82.450	1.00	
ATOM	266		PHE		42	8.190	73.248	82.402	1.00	
ATOM	267	CZ	PHE		42	9.111	72.443	83.100	1.00	
ATOM	268	N	LEU		43	6.790	71.375	77.765	1.00	
ATOM	269	CA	LEU		43	5.507	70.643	77.917	1.00	
ATOM	270	С	LEU		43	5.573	69.103	77.945	1.00	
	-					2.0.0	55.105		4.00	

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ATOM	271	0	LEU		43	4.957	68.410	78.749		17.69
MOTA	272	CB	LEU	A	43	4.472	71.003	76.826	1.00	21.60
ATOM	273	CG	LEU	A	43	3.213	71.850	77.034	1.00	24.67
MOTA	274	CD1	LEU	A	43	2.597	71.800	78.433	1.00	17.68
ATOM	275	CD2	LEU	A	43	2.172	71.549	75.953	1.00	21.72
ATOM	276	N	ASN	A	44	6.392	68.588	77.023	1.00	19.47
ATOM	277	CA	ASN	A	44	6.653	67.176	77.076	1.00	19.82
MOTA	278	C	ASN	A	44	7.419	66.619	78.312	1.00	17.44
MOTA	. 279	0	ASN	A	44	7.018	65.604	78.855	1.00	15.52
ATOM	280	CB	ASN	A	44	7.259	66.847	75.747	1.00	19.07
ATOM	281	CG	ASN	A	44	7.491	65.366	75.643	1.00	22.97
ATOM	282	OD1	ASN	A	44	8.605	64.906	75.468	1.00	30.35
ATOM	283	ND2	ASN	A	44	6.444	64.588	75.862	1.00	22.13
MOTA	284	N	ASP	A	45	8.482	67.324	78.726		19.71
ATOM	285	CA	ASP	A	45	9.175	67.050	80.020	1.00	19.87
ATOM	286	С	ASP	A	45	8.192	66.977	81.213	1.00	19.30
ATOM	287	0	ASP	A	45	8.103	66.009	81.956	1.00	21.00
ATOM	288	CB	ASP	A	45	10.225	68.119	80.273	1.00	13.57
ATOM	289	CG	ASP	A	45	11.563	67.769	79.706		12.64
ATOM	290	OD1	ASP		45	12.408	68.656	79.625		15.68
ATOM	291		ASP		45	11.823	66.611	79.414		14.57
ATOM	292	N	GLN		46	7.347	68.007	81.299		19.62
ATOM	293	CA	GLN		46	6.199	67.904	82.220		19.44
MOTA	294	С	GLN		46	5.259	66.702	82.166		22.23
ATOM	295	0	GLN		46	4.960	66.057	83.175		21.67
ATOM	296	CB	GLN		46	5.353	69.153	82.218		16.35
ATOM	297	CG	GLN		46	6.282	70.333	82.395		18.35
ATOM	298	CD	GLN		46	5.398	71.519	82.591		26.07
ATOM	299	OE1	GLN		46	5.334	72.143	83.629		31.83
ATOM	300	NE2			46	4.622	71.823	81.591		22.82
ATOM	301	N	ALA		47	4.838	66.364	80.935		19.12
ATOM	302	CA	ALA		47	3.979	65.187	80.813		17.83
ATOM	303	C	ALA		47	4.661	63.871	81.172		15.90
ATOM	304	ŏ	ALA		47	4.065	62.940	81.701		18.55
ATOM	305	CB	ALA		47	3.441	65.066	79.367		17.11
ATOM	306	N	MET		48	5.970		80.841		18.16
ATOM	307	CA	MET		48	6.799	63.818			
ATOM	308	C	MET		48	7.012	62.644	81.235 82.765		19.52 21.38
ATOM	309	o	MET				62.460			
		CB			48	6.996	61.358	83.316		20.83
ATOM	310		MET		48	8.173	62.667	80.539		21.42
MOTA	311	CG		A	48	8.150	62.603	78.984		29.81
ATOM	312 313	SD	MET		48	7.330	61.126	78.308		36.20
ATOM		CE	MET		48	5.582	61.633	78.280		33.60
ATOM	314	N	TYR		49	7.139	63.655	83.414		21.32
ATOM	315	CA	TYR		49	7.066	63.807	84.885		21.30
ATOM	316	C	TYR		49	5.773	63.244	85.515		22.58
ATOM	317	0	TYR		49	5.797	62.383	86.390		24.04
MOTA	318	CB	TYR		49	7.304	65.282	85.217		20.61
ATOM	319	CG	TYR		49	7.034	65.494	86.692		23.57
ATOM	320	CD1			49	5.755	65.931	87.109		23.57
ATOM	321		TYR		49	8.080	65.194	87.574		21.83
ATOM	322	CE1			49	5.524	66.097	88.481		26.09
ATOM	323		TYR		49	7.844	65.349	88.943		23.18
MOTA	324	CZ	TYR	A	49	6.591	65.842	89.377	1.00	26.31

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MOTA	325	ОН		R A		6.4	44 66.1	24 90.726	1.00	29.46
ATOM	326	N		JA		4.6	39 63.7	31 84.994	1.00	22.09
ATOM	327	CA	GL	JA	50	3.3	36 63.2	34 85.472	1.00	21.48
ATOM	328	C	GL	JA	50	3.0	52 61.7	76 85.230	1.00	23.20
ATOM	329	0	GLU	JA	50	2.5	48 61.0	50 86.081		24.23
ATOM	330	CB	GLU	JA	50	2.1	90 64.0	23 84.862	1.00	21.88
MOTA	331	CG	GLU	JA	50	2.3	04 65.5	37 84.986		21.13
ATOM	332	CD	GLU	JA	50	2.0	54 65.9°			25.41
ATOM	333	OE1	GLU	JA	50	1.8				24.65
ATOM	334	OE2			50	2.0				25.05
ATOM	335	N	GLIN	I A	51	3.4				23.02
ATOM	336	CA	GLN	A	51	3.4				24.72
ATOM	337	С	GLN	A	51	4.2				26.23
ATOM	338	0	GLN	A	51	3.8		_		25.10
ATOM	339	CB	GLN	I A	51	3.6				24.41
ATOM	340	CG	GLN	I A	51	3.4				38.05
ATOM	341	CD	GLN		51	2.1				53.15
ATOM	342	OE1	GLN	A	51	1.0				61.03
ATOM	343	NE2			51	2.0				55.24
ATOM	344	N	GLY		52	5.5				26.76
ATOM	345	CA	GLY		52	6.4				26.91
ATOM	346	C	GLY		52	5.7				25.60
ATOM	347	ō	GLY		52	5.6				24.37
ATOM	348	N	ARG		53	5.3				28.09
ATOM	349	CA	ARG		53	4.5				29.75
ATOM	350	c	ARG		53	3.3				
ATOM	351	ō	ARG		53	3.2				32.01
ATOM	352	СВ	ARG		53	4.1				34.69
ATOM	353	CG	ARG		53	3.8				30.41
ATOM	354	CD	ARG		53	3.5				29.15
ATOM	355	NE	ARG		53	2.3				29.38
ATOM	356	CZ	ARG		53					31.35
ATOM	357	NH1			53	1.0				32.29
ATOM	358	NH2				0.18				32.06
ATOM	359	N			53	0.60				32.66
ATOM	360	CA	LEU		54	2.57				32.47
ATOM	361	C	LEU		54	1.58				31.67
ATOM	362		LEU		54	2.11				33.30
ATOM		0	LEU		54	1.45				35.90
ATOM	363 364	CB	LEU		54	0.60				33.52
		CG	LEU		54	-0.59				32.85
ATOM	365	CD1			54	-1.29				30.53
ATOM	366		LEU		54	-0.28				36.56
ATOM	367	N	LEU		55	3.37	_			31.95
ATOM	368	CA	LEU		55	4.01			1.00	32.70
ATOM	369	С	LEU		55	4.57			1.00	33.98
ATOM	370	٥	LEU		55	4.84			1.00	32.73
ATOM	371	CB	LEU		55	5.19				31.04
ATOM	372	CG	LEU		55	4.83				28.60
ATOM	373		LEU		55	3.96		7 85.101		27.88
ATOM	374		LEU		55	6.11		L 84.539		28.33
ATOM	375	N	ARG		56	4.73		89.911	1.00	
ATOM	376	CA	ARG		56	5.25	7 55.30	91.281	1.00	
ATOM	377	С	ARG		5 6	4.61	6 54.240	92.164	1.00	
MOTA	378	0	ARG	A	56	5.26	0 53.518		1.00	

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				_		5 240	EC (12	91.993	1.00 32.58
ATOM	379	CB	ARG		56	5.249	56.643		
ATOM	380	CG	ARG	A	56	6.368	57.502	91.476	1.00 22.04
ATOM	381	CD	ARG	A	56	6.142	58.874	92.049	1.00 21.74
ATOM	382	NE	ARG	A	56	7.073	59.804	91.447	1.00 23.56
ATOM	383	CZ	ARG	A	56	7.062	61.074	91.750	1.00 25.56
ATOM	384	NH1	ARG	A	56	6.401	61.444	92.790	1.00 30.94
ATOM	385		ARG		56	7.688	61.979	91.035	1.00 22.33
ATOM	386	N	ASN		57	3.306	54.120	91.997	1.00 44.42
						2.602	53.027	92.680	1.00 48.62
ATOM	387	CA	ASN		57				
ATOM	388	С	ASN		57	2.786	51.585	92.169	1.00 47.46
ATOM	389	0	ASN		57	2.316	50.630	92.759	1.00 51.15
MOTA	390	CB	ASN	A	57	1.124	53.435	92.726	1.00 59.30
MOTA	391	CG	ASN	A	57	0.389	53.137	94.049	1.00 68.98
MOTA	392	OD1	ASN	A	57	-0.829	53.335	94.164	1.00 75.09
ATOM	393	ND2	ASN	A	57	1.140	52.692	95.058	1.00 71.68
ATOM	394	N	THR	A	58	3.461	51,442	91.036	1.00 42.68
ATOM	395	CA	THR		58	3.555	50.086	90.475	1.00 36.64
ATOM	396	C	THR		58	4.821	49.318	90.871	1.00 33.64
			THR		58	5.721	49.876	91.477	1.00 31.69
ATOM	397	0						88.948	1.00 36.81
ATOM	398	CB	THR		58	3.492	50.189		
MOTA	399	OG1	THR		58	4.774	50.576	88.447	1.00 37.64
ATOM	400	CG2	THR		58	2.432	51.203	88.507	1.00 35.36
ATOM	401	N	GLU		59	4.937	48.068	90.409	1.00 33.08
MOTA	402	CA	GLU	A	59	6.238	47.410	90.581	1.00 34.80
ATOM	403	С	GLU	A	59	7.487	48.104	89.944	1.00 33.45
ATOM	404	0	GLU	A	59	8.607	48.153	90.463	1.00 34.28
MOTA	405	CB	GLU	A	59	6.067	45.933	90.191	1.00 43.43
ATOM	406	CG	GLU	A	59	7.242	45.007	90.614	1.00 59.74
ATOM	407	CD	GLU	A	59	7.519	44.933	92.159	1.00 69.61
ATOM	408	OE1	GLU		59	6.582	45.064	92.960	1.00 74.78
ATOM	409	OE2	GLU		59	8.686	44.751	92.589	1.00 74.97
MOTA	410	N	ARG		60	7.229	48.734	88.768	1.00 27.61
	411	CA	ARG		60	8.251	49.599	88.158	1.00 25.02
MOTA								88.958	1.00 22.94
MOTA	412	C	ARG		60	8.614	50.851		
ATOM	413	0	ARG		60	9.772	51.257	89.002	1.00 24.63
ATOM	414	CB	ARG		60	7.874	49.966	86.690	1.00 26.16
ATOM	415	CG	ARG	A	60	8.877	50.860	85.900	1.00 24.47
MOTA	416	CD	ARG	A	60	10.268	50.249	85.758	1.00 23.96
ATOM	417	NE	ARG	A	60	11.285	51.161	85.217	1.00 25.64
ATOM	418	CZ	ARG	A	60	12.214	51.778	85.945	1.00 24.77
MOTA	419	NH1	ARG	A	60	12.159	51.805	87.261	1.00 24.78
ATOM	420	NH2	ARG	A	60	13.227	52.294	85.325	1.00 19.79
ATOM	421	N	GLY	A	61	7.562	51.411	89.587	1.00 21.94
ATOM	422	CA	GLY	A	61	7.623	52.443	90.620	1.00 22.33
ATOM	423	С	GLY		61	8.468	52.051	91.824	1.00 24.44
ATOM	424	ō	GLY		61	9.350	52.773	92.253	1.00 25.22
		_					50.821	92.307	1.00 26.95
MOTA	425	N	LYS		62	8.248 9.102		93.350	1.00 26.24
ATOM	426	CA	LYS		62		50.251		1.00 24.89
MOTA	427	C	LYS		62	10.590	50.158	93.045	
MOTA	428	0	LYS		62	11.443	50.668	93.756	1.00 23.23
MOTA	429	CB	LYS		62	8.519	48.900	93.723	1.00 29.90
MOTA	430	CG	LYS		62	9.379	48.296	94.835	1.00 38.76
MOTA	431	CD	LYS		62	8.847	46.904	95.222	1.00 47.47
MOTA	432	CE	LYS	A	62	9.944	45.971	95.773	1.00 53.72

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ATOM	433	NZ	LYS	A	62	10.167	44.857	94.832	1.00	60.22
MOTA	434	N	LEU	JA	63	10.866	49.560	91.882	1.00	24.67
ATOM	435	CA	LEU	JA	63	12.239	49.634	91.346	1.00	23.65
ATOM	436	С	LEU		63	12.805	51.043	91.186	1.00	22.74
ATOM	437	0	LEU		63	13.927	51.359	91.517		25.19
ATOM	438	CB	LEU		63	12.232	48.973	89.981	1.00	27.11
ATOM	439	CG	LEU		63	13.477	48.298	89.403	1.00	31.83
MOTA	440	CD1			63	14.808	48.658	90.077		33.84
ATOM	441	CD2			63	13.440	48.410	87.874	1.00	26.95
ATOM	442	N	ALA		64	11.979	51.940	90.642	1.00	21.62
ATOM	443	CA	ALA		64	12.492	53.308	90.539		20.51
ATOM	444	C	ALA		64	12.862	53.971	91.863		21.79
ATOM	445	0	ALA		64	13.890	54.636	91.984		21.21
ATOM	446	CB	ALA		64	11.456	54.196	89.862		19.01
ATOM ATOM	447	N	ALA		65	11.984	53.747	92.870		23.25
	448	CA	ALA		65	12.374	54.212	94.235		24.88
ATOM ATOM	449	C	ALA		65	13.684	53.619	94.784		22.62
ATOM	450 451	O CB	ALA		65 CE	14.551	54.328	95.268		22.95
ATOM	452	N	ALA GLU		65 66	11.249	54.013	95.265		25.38
ATOM	453	CA	GLU		66 66	13.848	52.295	94.572		23.38
ATOM	454	C	GLU		66	15.116 16.332	51.632	94.878		23.41
ATOM	455	ō	GLU		66	17.321	52.188 52.604	94.152		26.06
ATOM	456	СВ	GLU		66	14.968	50.136	94.744		25.12
ATOM	457	CG	GLU		66	13.818	49.616	94.665 95.533		25.85
ATOM	458	CD	GLU		66	13.546	48.142	95.293		31.94 37.59
ATOM	459	OE1			66	13.147	47.430	96.220		40.33
ATOM	460	OE2			66	13.721	47.673	94.176		40.79
ATOM	461	N	ASP		67	16.204	52.276	92.817		24.01
ATOM	462	CA	ASP		67	17.222	52.928	91.986		19.72
ATOM	463	C	ASP		67	17.549	54.333	92.402		16.72
ATOM	464	0	ASP		67	18.694	54.767	92.414		18.91
ATOM	465	CB	ASP		67	16.787	52.944	90.495		21.68
ATOM	466	CG	ASP		67	16.824	51.580	89.801		25.22
ATOM	467		ASP		67	17.340	50.629	90.370		23.32
ATOM	468	OD2	ASP	A	67	16.349	51.434	88.666		26.83
ATOM	469	N	ALA	A	68	16.485	55.059	92.773		16.48
ATOM	470	CA	ALA	A	68	16.685	56.425	93.250		19.28
MOTA	471	C	ALA		68	17.489	56.510	94.569		20.86
ATOM	472	0	ALA	A	68	18.165	57.494	94.837		22.07
ATOM	473	CB	ALA	A	68	15.330	57.134	93.419		19.81
MOTA	474	N	ASN	A	69	17.472	55.371	95.299		23.11
ATOM	475	CA	ASN	A	69	18.330	55.262	96.514		27.41
ATOM	476	C	ASN	A	69	19.816	55.042	96.273		29.49
ATOM	477	0	ASN	A	69	20.646	55.304	97.140		28.64
ATOM	478	СВ	ASN	A	69	17.933	54.145	97.466	1.00	24.19
ATOM	479	CG	ASN	A	69	16.632	54.425	98.142	1.00	25.79
ATOM	480		ASN		69	16.298	55.549	98.445		26.84
ATOM	481	ND2	ASN	A	69	15.894	53.359	98.410		30.16
ATOM	482	N	LEU		70	20.104	54.574	95.034		25.30
ATOM	483	CA	LEU		70	21.514	54.442	94.627		23.20
ATOM	484	C	LEU		70	22.329	55.691	94.640		21.41
ATOM	485	0	LEU		70	22.013	56.696	94.028		23.50
ATOM	486	CB	LEU	A	70	21.672	53.890	93.225	1.00	22.19

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487 488 490 491 492 493 495 496 497 498 5001 5002 5005 5007 5008 5009 511	CG CD1 CD2 N CA C O CB OG N CA C O CB OG N CA C O CB OG N CA C O C O C O C O O O O O O O O O O O	LEU SER SER SER SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A	70 70 70 71 71 71 71 72 72 72 72 72 73 73 73 73 74	21.078 21.830 21.016 23.450 24.527 25.355 25.269 25.453 26.230 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	52.512 51.459 52.154 55.563 56.515 56.171 55.081 56.521 55.303 57.079 54.663 57.834 58.966 55.318 54.068 52.783 51.705	93.095 93.896 91.624 95.304 95.119 93.888 93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
489 491 492 493 494 495 496 497 498 5001 5002 5004 5005 5007 5008 5009 511	CD2 N CA C O CB OG N CA C O CB OG N CA C O N	LEU SER SER SER SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A	70 71 71 71 71 72 72 72 72 72 72 73 73 73 73	21.016 23.450 24.527 25.355 25.269 25.453 26.232 26.220 27.096 27.860 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	52.154 55.563 56.515 56.171 55.081 56.521 55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	91.624 95.304 95.119 93.888 93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	23.47 21.73 22.25 20.52 23.70 22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
490 491 492 493 494 495 496 497 498 500 501 502 503 504 505 507 508 509 510	CACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	SER SER SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A	71 71 71 71 71 72 72 72 72 72 72 73 73 73	23.450 24.527 25.355 25.269 25.453 26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	55.563 56.515 56.171 55.081 56.521 55.303 57.079 56.747 55.4663 57.834 58.966 55.318 54.068 52.783	95.304 95.119 93.888 93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	21.73 22.25 20.52 23.70 22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
491 493 494 495 496 497 498 500 501 502 503 504 505 506 507 508 509 510	CA C O CB OG N CA C O CB OG N CA C O N CA C O N CA	SER SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A A A A	71 71 71 71 72 72 72 72 72 72 73 73 73 73	24.527 25.355 25.269 25.453 26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	56.515 56.171 55.081 56.521 55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	95.119 93.888 93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	22.25 20.52 23.70 22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510	C CB OG CA C C O N CA C C O	SER SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A	71 71 71 72 72 72 72 72 72 73 73 73 73	25.355 25.269 25.453 26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	56.171 55.081 56.521 55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	93.888 93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	20.52 23.70 22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510	O CB OG CA C O N CA C O N CA C O O C C C O O C C C O O C C C C O O C C C C O C C C O C C C O C C C O C C C C O C C C C O C C C O C C C C O C	SER SER SER SER SER SER GLY GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A	71 71 72 72 72 72 72 72 72 73 73 73 73	25.269 25.453 26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	55.081 56.521 55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	93.357 96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00	23.70 22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510	CB OG N CA C O CB OG N CA C O CA C	SER SER SER SER SER SER GLY GLY GLY GLY GLY	A A A A A A A A A A A A A A A A A	71 71 72 72 72 72 72 72 73 73 73 73	25.453 26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	56.521 55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	96.349 96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00	22.74 28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510	OG N CA C O N CA C O	SER SER SER SER SER GLY GLY GLY GLY GLY GLY	A A A A A A A A A A A A A	71 72 72 72 72 72 72 73 73 73 73	26.232 26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	55.303 57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	96.432 93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00 1.00	28.68 20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
496 497 498 499 500 501 502 503 504 505 506 507 508 509 510	CA CC CB CB CC CA CC CC CC	SER SER SER SER SER GLY GLY GLY GLY GLY GLY	A A A A A A A	72 72 72 72 72 72 73 73 73 73	26.220 27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	57.079 56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	93.445 92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00	20.02 20.88 22.52 21.65 19.05 22.00 20.71 16.81
497 498 499 500 501 502 503 504 505 506 507 508 509 510	CA C O CB OG N CA C O N CA	SER SER SER SER GLY GLY GLY GLY GLY GLY	A A A A A A	72 72 72 72 72 73 73 73 73	27.096 27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	56.747 55.479 54.663 57.834 58.966 55.318 54.068 52.783	92.294 92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00 1.00	20.88 22.52 21.65 19.05 22.00 20.71 16.81
498 499 500 501 502 503 504 505 506 507 508 509 510	C O CB OG N CA C O	SER SER SER GLY GLY GLY GLY GLY GLY	A A A A A A	72 72 72 72 73 73 73 73	27.860 27.979 28.113 27.352 28.336 28.979 28.146 28.697	55.479 54.663 57.834 58.966 55.318 54.068 52.783	92.410 91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00 1.00	22.52 21.65 19.05 22.00 20.71 16.81
499 500 501 502 503 504 505 506 507 508 509 510	O CB OG N CA C O C	SER SER GLY GLY GLY GLY GLY GLY	A A A A A A	72 72 72 73 73 73 73	27.979 28.113 27.352 28.336 28.979 28.146 28.697	54.663 57.834 58.966 55.318 54.068 52.783	91.518 92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00	21.65 19.05 22.00 20.71 16.81
500 501 502 503 504 505 506 507 508 509 510	CB OG N CA C O N CA C	SER GLY GLY GLY GLY GLY GLY	A A A A A	72 72 73 73 73 73	28.113 27.352 28.336 28.979 28.146 28.697	57.834 58.966 55.318 54.068 52.783	92.083 91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00	19.05 22.00 20.71 16.81
501 502 503 504 505 506 507 508 509 510	OG N CA C O N CA C	SER GLY GLY GLY GLY GLY	A A A A A	72 73 73 73 73	27.352 28.336 28.979 28.146 28.697	58.966 55.318 54.068 52.783	91.735 93.640 94.006 93.939	1.00 1.00 1.00 1.00	19.05 22.00 20.71 16.81
502 503 504 505 506 507 508 509 510	N CA C O N CA C	GTA GTA GTA GTA GTA GTA	A A A A	73 73 73 73	28.336 28.979 28.146 28.697	55.318 54.068 52.783	91.735 93.640 94.006 93.939	1.00 1.00	22.00 20.71 16.81
503 504 505 506 507 508 509 510	CA C O II CA C	GTA GTA GTA GTA GTA	A A A A	73 73 73	28.979 28.146 28.697	55.318 54.068 52.783	93.640 94.006 93.939	1.00 1.00	20.71 16.81
504 505 506 507 508 509 510	C N CA C	GTA GTA GTA GTA	A A A	73 73	28.979 28.146 28.697	54.068 52.783	94.006 93.939	1.00	16.81
504 505 506 507 508 509 510	C N CA C	GTA GTA GTA GTA	A A A	73 73	28.146 28.697	52.783	93.939		
505 506 507 508 509 510 511	O N CA C	GLY GLY GLY	A A	73	28.697				15.97
506 507 508 509 510 511	N CA C	GLY GLY	A				93.753	1.00	
507 508 509 510 511	CA C O	GLY GLY			26.818	52.915	94.046		16.07
508 509 510 511	0	GLY		74	26.090	51.649	93.967	1.00	
509 510 511	0		Α	74	25.671	51.260	92.526		21.98
510 511		GLY		74	25.202	50.164	92.238		21,28
511		VAL		75	25.887	52.210	91.567		22.48
_	CA	VAL		75	25.521	51.777	90.174		22.71
512	C	VAL		75	26.174	50.493	89.628		18.50
513	0	VAL		75	25.497	49.573	89.210		20.32
514	CB	VAL		75	25.820	52.946	89.218		23.52
									21.57
									17.23
									18.62
									19.92
534	CB	ALA		78 70	22.636	49.160	90.208		13.62
FOF	N	PHE		79 70	24.025	46.748	88.292		19.22
535		PHE		79 70	24.019	45.921	87.070		20.71
536	CA	PHE		79 70	24.117	44.420	87.238	1.00	23.30
536 537	C		A	79	24,161	43.662	86.273		
536 537 538	c o	PHE	~	70	05 4			1.00	
536 537	C			79 79	25.116 24.821	46.352 47.683	86.082 85.382	1.00	23.64 18.00 19.59
	515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533	515 CG1 516 CG2 517 N 518 CA 519 C 520 O 521 CB 522 N 523 CA 524 C 525 O 526 CB 527 CG 528 OD1 529 ND2 530 N 531 CA 532 C	515 CG1 VAL 516 CG2 VAL 517 N ALA 518 CA ALA 519 C ALA 520 O ALA 521 CB ALA 522 N ASN 523 CA ASN 524 C ASN 525 O ASN 526 CB ASN 526 CB ASN 527 CG ASN 527 CG ASN 528 OD1 ASN 529 ND2 ASN 529 ND2 ASN 530 N ALA 531 CA ALA 532 C ALA	515 CG1 VAL A 516 CG2 VAL A 517 N ALA A 518 CA ALA A 519 C ALA A 520 O ALA A 521 CB ALA A 522 N ASN A 523 CA ASN A 524 C ASN A 525 O ASN A 526 CB ASN A 527 CG ASN A 527 CG ASN A 528 OD1 ASN A 529 ND2 ASN A 529 ND2 ASN A 530 N ALA A 531 CA ALA A 532 C ALA A	515 CG1 VAL A 75 516 CG2 VAL A 75 517 N ALA A 76 518 CA ALA A 76 519 C ALA A 76 520 O ALA A 76 521 CB ALA A 76 522 N ASN A 77 523 CA ASN A 77 524 C ASN A 77 525 O ASN A 77 526 CB ASN A 77 526 CB ASN A 77 527 CG ASN A 77 527 CG ASN A 77 528 OD1 ASN A 77 529 ND2 ASN A 77 529 ND2 ASN A 77 530 N ALA A 78 531 CA ALA A 78 532 C ALA A 78	515 CG1 VAL A 75 25.719 516 CG2 VAL A 75 25.153 517 N ALA A 76 27.517 518 CA ALA A 76 28.149 519 C ALA A 76 27.414 520 O ALA A 76 27.033 521 CB ALA A 76 29.612 522 N ASN A 77 27.131 523 CA ASN A 77 26.463 524 C ASN A 77 25.019 525 O ASN A 77 24.536 526 CB ASN A 77 26.615 527 CG ASN A 77 25.817 528 OD1 ASN A 77 25.817 528 OD1 ASN A 77 25.817 529 ND2 ASN A 77 26.435 530 N ALA A 78 24.377 531 CA ALA A 78 23.060 532 C ALA A 78 22.874	515 CG1 VAL A 75 25.719 52.707 516 CG2 VAL A 75 25.153 54.265 517 N ALA A 76 27.517 50.394 518 CA ALA A 76 28.149 49.125 519 C ALA A 76 27.414 47.875 520 O ALA A 76 27.033 47.028 521 CB ALA A 76 29.612 49.071 522 N ASN A 77 27.131 47.820 523 CA ASN A 77 26.463 46.601 524 C ASN A 77 25.019 46.464 525 O ASN A 77 24.536 45.350 526 CB ASN A 77 24.536 45.350 526 CB ASN A 77 26.615 46.509 527 CG ASN A 77 25.817 45.362 528 OD1 ASN A 77 24.672 45.508 529 ND2 ASN A 77 26.435 44.202 530 N ALA A 78 24.377 47.638 531 CA ALA A 78 23.060 47.710 532 C ALA A 78 22.874 47.025 533 O ALA A 78 22.874 47.025	515 CG1 VAL A 75	515 CG1 VAL A 75

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ATOM	541		PHE		79	25.181	48.903	85.984		17.05
MOTA	542	CD2			79	24.214	47.687	84.104		18.56
ATOM	543	CE1			79	25.026	50.119	85.298		19.06
ATOM	544	CE2			79	24.040	48.906	83.419		15.97
ATOM	545	CZ	PHE		79	24.500	50.104	83.989		17.87
ATOM	546	N	SEF		80	24.150	43.947	88.488	1.00	19.74
MOTA	547	CA	SER		80	24.500	42.511	88.632	1.00	17.29
ATOM	548	С	SER	A	80	23.601	41.501	87.944	1.00	18.57
ATOM	549	0	SER	A	80	23.977	40.494	87.339	1.00	22.38
ATOM	550	CB	SER	A	80	24.608	42.106	90.125	1.00	15.17
ATOM	551	OG	SER	A	80	25.646	42.920	90.700	1.00	17.32
ATOM	552	N	GLY	Α	81	22.309	41.852	88.041	1.00	19.68
ATOM	553	CA	GLY	Ά	81	21.271	41.005	87.413	1.00	22.50
ATOM	554	C	GLY	A	81	21.293	40.977	85.855	1.00	24.85
ATOM	555	0	GLY	A	81	21.318	39.939	85.211	1.00	23.46
ATOM	556	N	ALA	A	82	21.380	42.197	85.279	1.00	24.60
ATOM	557	CA	ALA	A	82	21.686	42.339	83.855		24.50
ATOM	558	C	ALA	A	82	22.985	41.643	83.417	1.00	25.22
ATOM	559	0	ALA	A	82	23.000	40.873	82.468	1.00	23.42
ATOM	560	CB	ALA	A	82	21.649	43.819	83.470	1.00	20.35
ATOM	561	N	PHE	A	83	24.050	41.874	84.197		23.76
ATOM	562	CA	PHE	A	83	25.319	41.242	83.894		21.76
ATOM	563	С	PHE	A	83	25.325	39.726	83.974		22.85
ATOM	564	0	PHE	A	83	26.090	39.052	83.322		25.72
ATOM	565	CB	PHE	A	83	26.349	41.867	84.792		20.01
ATOM	566	CG	PHE	A	83	27.770	41.527	84.394		20.95
ATOM	567	CD1	PHE	A	83	28.486	40.587	85.152		16.94
ATOM	568	CD2	PHE		83	28.391	42.208	83.307		23.09
ATOM	569		PHE	A	83	29.841	40.355	84.843		18.79
ATOM	570		PHE		83	29.751	41.991	83.001		20.95
ATOM	571	CZ	PHE		83	30.474	41.069	83.795		21.43
ATOM	572	N	GLY		84	24.409	39.187	84.768		22.31
ATOM	573	CA	GLY		84	24.478	37.740	84.865		24.39
MOTA	574	С	GLY		84	25.199	37.163	86.093		29.49
ATOM	575	0	GLY		B4	25.158	35.963	86.362		32.27
ATOM	576	N	SER		85	25.873	38.058	86.843		30.33
ATOM	577	ÇA	SER		85	26.685	37.625	88.001		28.46
ATOM	578	C	SER		85	27.047	38.788	88.936		29.06
ATOM	579	ō	SER		85	26.915	39.945	88.556		29.00
ATOM	580	СВ	SER		85	27.915	36.861	87.536		24.04
ATOM	581	OG	SER		85	28.903	37.746	87.028	1.00	
ATOM	582	N	PRO		86	27.436	38.518	90.216		29.45
ATOM	583	CA	PRO		86	27.599	39.650	91.122	1.00	
ATOM	584	C	PRO		86	28.721	40.513	90.733		
ATOM	585	ō	PRO		86	29.830	40.064	90.733	1.00	22.75
ATOM	586	СВ	PRO		86	27.873	39.029	92.493	1.00	
ATOM	587	CG		_						
ATOM	588	CD	PRO		86 86	27.284 27.591	37.627 37.258	92.399 90.945	1.00	
ATOM	589	N	ILE		87	28.350			1.00	
ATOM	590	CA	ILE		87	29.363	41.776	90.659	1.00	
ATOM	591	C	ILE		87	29.363	42.816	90.469	1.00	
MOTA	592	0	ILE		87	28.956	43.494	91.811	1.00	
ATOM	593	СВ	ILE				44.424	92.220	1.00	
ATOM	594		ILE		87	28.908	43.860	89.427	1.00	
AIUM	334	CGT	TTE	A	87	28.626	43.165	88.076	1.00	23.29

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ATOM	595	CG2	ILE	A	87	29.997	44.923	89.306	1.00	23.62
ATOM	596	CD1	ILE		87	27.925	44.100	87.092		21.95
MOTA	597	M	THR	A	88	30.655	42.914	92.481	1.00	25.86
ATOM	598	CA	THR	A	88	30.925	43.247	93.903	1.00	25.48
ATOM	599	С	THR	A	88	32.418	43.249	94.166	1.00	25.25
ATOM	600	0	THR	A	88	33.131	42.561	93.445	1.00	24.26
ATOM	601	CB	THR	A	88	30.332	42.211	94.859		22.31
ATOM	602	OG1	THR	A	88	31.102	41.029	94.702	1.00	25.42
ATOM	603	CG2	THR	A	88	28.833	41.943	94.710	1.00	19.45
ATOM	604	N	GLU	A	89	32.891	43.970	95.204	1.00	26.63
MOTA	605	CA	GLU	A	89	34.322	43.845	95.577	1.00	25.29
ATOM	606	С	GLU	A	89	34.810	42.429	95.889	1.00	25.30
ATOM	607	0	GLU	A	89	35.924	41.999	95.611	1.00	26.30
MOTA	608	CB	GLU	A	89	34.652	44.773	96.741	1.00	25.64
MOTA	609	CG	GLU	A	89	34.334	46.193	96.340	1.00	26.52
MOTA	610	CD	GLU	A	.89	34.551	47.228	97.414	1.00	29.70
ATOM	611	OE1	GLU	A	89	35.136	48.245	97.123	1.00	33.05
MOTA	612	OE2	GLU	A	89	34.138	47.077	98.540	1.00	27.59
ATOM	613	N	LYS	A	90	33.860	41.697	96.459	1.00	26.25
ATOM	614	CA	LYS	A	90	34.095	40.310	96.883	1.00	28.81
ATOM	615	С	LYS	A	90	34.285	39.313	95.780	1.00	28.19
ATOM	616	0	LYS	A	90	35.206	38.518	95.773	1.00	30.49
ATOM	617	CB	LYS	A	90	32.889	39.869	97.672	1.00	31.00
ATOM	618	CG	LYS	A	90	32.956	38.478	98.228	1.00	37.00
ATOM	619	CD	LYS	A	90	31.536	38.026	98.583	1.00	43.53
MOTA	620	CE	LYS	A	90	31.386	36.504	98.712	1.00	50.17
ATOM	621	NZ	LYS	A	90	32.257	35.875	97.701	1.00	60.80
MOTA	622	N	ASP	A	91	33.324	39.416	94.870	1.00	28.68
ATOM	623	CA	ASP	A	91	33.271	38.504	93.738		29.34
ATOM	624	С	ASP	A	91	33.911	38.947	92.420	1.00	29.37
ATOM	625	0	ASP	A	91	34.429	38.173	91.635	1.00	31.54
ATOM	626	СВ	ASP	A	91	31.827	38.162	93.438	1.00	30.91
ATOM	627	CG	ASP	A	91	31.087	37.722	94.674	1.00	31.69
ATOM	628	OD1	ASP	A	91	31.395	36.657	95.212		35.66
ATOM	629	OD2	ASP	A	91	30.186	38.438	95.088	1.00	29.63
ATOM	630	N	ALA		92	33.830	40.240	92.165	1.00	28.62
ATOM	631	CA	ALA	A	92	34.443	40.713	90.919		26.75
ATOM	632	С	ALA	A	92	35.255	41.981	91.131		26.94
MOTA	633	0	ALA	A	92	34.937	43.081	90.690	1.00	26.94
ATOM	634	CB	ALA	A	92	33.390	40.934	89.817		23.93
ATOM	635	N	PRO	A	93	36.336	41,826	91.930		27.16
ATOM	636	CA	PRO	A	93	37.151	43.015	92.274	1.00	
ATOM	637	С	PRO		93	37.832	43.865	91.160		24.62
ATOM	638	0	PRO	A	93	37.844	45.098	91.178	1.00	22.75
ATOM	639	СВ	PRO		93	38.120	42.411	93.292		25.31
ATOM	640	CG	PRO		93	38.219	40.926	92.945		23.54
ATOM	641	CD	PRO		93	36.817	40.578	92.534		25.29
ATOM	642	N	ALA		94	38.409	43.174	90.170		25.16
ATOM	643	CA	ALA		94	38.954	43.886	89.005		23.77
ATOM	644	c	ALA		94	37.923	44.715	88.249		17.72
ATOM	645	ō	ALA		94	38.116	45.897	88.005		19.91
ATOM	646	СВ	ALA		94	39.676	42.931	88.058		21.52
ATOM	647	N	LEU		95	36.787	44.081	B8.026		19.68
ATOM	648	CA	LEU		95	35.577	44.770	87.539		20.23
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ATOM	649	С	LEU	I A	. 95	35.001	45.902	88.385	1.00	21.85
ATOM	650	0	LEU	A	. 95	34.809	47.026	87.943	1.00	21.66
MOTA	651	CB	LEU	A	. 95	34.466	43.755	87.271	1.00	20.70
ATOM	652	CG	LEU	A	95	33.250	44.285	86.467	1.00	21.32
ATOM	653	CD1	LEU	A	. 95	32.299	43.149	86.063	1.00	20.10
ATOM	654	CD2	LEU	Α	95	33.698	45.111	85.240	1.00	20.93
ATOM	655	N	HIS	Α	. 96	34.755	45.606	89.667	1.00	21.70
ATOM	656	CA	HIS	A	96	34.313	46.691	90.543		17.84
ATOM	657	C	HIS	A	96	35.214	47,924	90.580	1.00	17.76
ATOM	658	0	HIS	A	96	34.767	49.069	90.496	1.00	19.44
ATOM	659	CB	HIS	A	96	34.042	46.116	91.937	1.00	20.62
ATOM	660	CG	HIS	A	96	32.934	46.893	92.613	1.00	21.96
ATOM	661	ND1	HIS	A	96	33.021	48.094	93.229		21.34
ATOM	662	CD2	HIS	A	96	31.614	46.470	92.680	1.00	24.62
MOTA	663	CEl	HIS	A	96	31.790	48.442	93.690		18.16
MOTA	664	NE2	HIS	A	96	30.923	47.437	93.338		23.59
MOTA	665	N	LYS	A	97	36.539	47.639	90.629		18.11
ATOM	666	CA	LYS	A	97	37.544	48.713	90.581		18.50
MOTA	667	C	LYS	A	97	37.519	49.564	89.317		20.80
ATOM	668	0	LYS	A	97	37.452	50.781	89.373		21.08
ATOM	669	CB	LYS		97	38.924	48.085	90.766		17.26
ATOM	670	CG	LYS		97	40.125	49.014	90.594		21.24
ATOM	671	CD	LYS		97	40.283	50.213	91.525		30.10
ATOM	672	CE	LYS		97	41.482	51.160	91.164		33.55
ATOM	673	NZ	LYS		97	41.557	52.449	91.915		29.55
ATOM	674	N	LEU		98	37.532	48.857	88.155		22.36
ATOM	675	CA	LEU		98	37.291	49.555	86.851		22.04
ATOM	676	С	LEU		98	36.128	50.581	86.806		17.59
ATOM	677	0	LEU		98	36.223	51.763	86.522		18.13
ATOM	678	CB	LEU		98	37.025	48.477	85.780		21.44
ATOM	679	CG	LEU		98	36.766	49.042	84.375		20.93
ATOM	680	CD1			98	36.265	47.902	83.493		22.92
MOTA	681				98	37.963	49.801	83.811		18.27
ATOM	682	N	LEU		99	34.977	50.024	87.188		19.16
ATOM	683	CA	LEU		99	33.753	50.802	87.186		18.37
ATOM	684	c	LEU		99	33.644	51.930	88.169		20.61
ATOM	685	ō	LEU		99	33.068	52.964	87.883		18.31
ATOM	686	СВ	LEU		99	32.545	49.874	87.263		18.90
ATOM	687	CG	LEU		99	32.428	48.860	86.191		20.87
ATOM	688	CD1	LEU		99	32.464	49.503	84.841		14.73
ATOM	689	CD2	LEU		99	31.190	48.099	86.497		19.82
ATOM	690	N			100	34.252	51.692	89.359		22.03
ATOM	691	CA			100	34.357	52.777	90.360		
ATOM	692	C			100	35.259	53.938	89.957		19.41
ATOM	693	o			100	34.984	55.118			16.72
ATOM	694	СВ			100	34.889	52.174	90.136 91.674		16.55
ATOM	695		THR							19.50
ATOM	696		THR			34.030 35.092	51.113	92.091	1.00	
ATOM	697	N N			101		53.221	92.758	1.00	
ATOM	698	CA			101	36.397 37.415	53.537	89.358	1.00	
ATOM	699	CA	ASN				54.515	88.998	1.00	
	700	0				37.022	55.479	87.873	1.00	
ATOM ATOM	701		ASN			37.610	56.546	87.711	1.00	
		CB	ASN			38.692	53.763	88.716	1.00	
ATOM	702	CG	ASN	A	TAT	39.950	54.556	89.041	1.00	22.36

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2001				_		40 000				
MOTA	703		ASN			40.938	53.978	89.459		30.43
ATOM	704	ND2				39.964	55.868	88.889	1.00	23.45
ATOM	705	N	MET	A	102	35.952	55.090	87.154	1.00	21.94
ATOM	706	CA	MET	A	102	35.407	55.984	86.103	1.00	22.28
ATOM	707	С	MET	A	102	34.142	56.755	86.455	1.00	22.36
ATOM	708	0			102	33.571	57.482	85.638		23.37
ATOM	709	СВ			102	35.162	55.213	84.781		19.03
ATOM	710	CG			102	34.239	54.001	84.972		18.05
	711									
ATOM		SD			102	33.744	53.082	83.481		20.07
ATOM	712	CE			102	32.429	54.165	83.010		16.04
ATOM	713	N			103	33.681	56.555	87.724		21.00
ATOM	714	CA			103	32.441	57.221	88.180		18.78
ATOM	715	С			103	32.371	58.697	87.833		17.38
ATOM	716	0	ILE	A	103	31.413	59.152	87.245	1.00	18.08
ATOM	717	CB	ILE	A	103	32.174	57.025	89.732	1.00	16.18
MOTA	718	CG1	ILE	A	103	31.696	55.603	90.031	1.00	19.21
ATOM	719	CG2	ILE	A	103	31.135	58.037	90.272	1.00	12.63
ATOM	720	CD1	ILE	A	103	31.708	55.185	91.522	1.00	17.58
ATOM	721	N	GLU	A	104	33,426	59.429	88.218		18.81
ATOM	722	CA	GLU	A	104	33.369	60.900	88.092		18.36
ATOM	723	С			104	33.828	61.520	86.772		19.60
ATOM	724	o			104	33.420	62.606	86.365		19.13
ATOM	725	CB			104	34.092	61.600	89.241		18.56
ATOM	726	CG			104	33.446	61.448	90.617		19.21
ATOM	727	CD								23.36
	728				104	31.994	61.944	90.665		
ATOM			GLU			31.225	61.359	91.382		26.94
ATOM	729	OE2			104	31.574	62.888	90.013		28.46
ATOM	730	N			105	34.606	60.713	86.049		18.95
MOTA	731	CA			105	34.743	60.936	84.587		17.40
ATOM	732	С			105	33.378	61.099	83.886		15.12
ATOM	733	0	ASP	A	105	33.104	62.102	83.234	1.00	18.61
ATOM	734	CB	ASP	A	105	35.429	59.743	83.951	1.00	16.81
ATOM	735	CG	ASP	A	105	36.831	59.545	84.440	1.00	15.10
ATOM	736	QD1	ASP	A	105	37.573	60.520	84.573	1.00	19.01
MOTA	737	OD2	ASP	A	105	37.177	58.402	84.685	1.00	15.56
ATOM	738	N			106	32.500	60.091	84.096		15.05
ATOM	739	CA			106	31.111	60.157	B3.607		15.61
ATOM	740	С	ALA			30.166	61.126	84.315		19.62
ATOM	741	ō	ALA			29.409	61.881	83.720		18.83
ATOM	742	СВ			106	30.467	58.782	83.682		11.73
ATOM	743	N	GLY			30.263	61.102	85.674		21.49
ATOM	744	CA	GLY							
						29.323	61.899	86.503		16.83
ATOM	745	C	GLY			29.599	63.356	86.594		14.80
ATOM	746	0	GLY			28.714	64.204	86.575		17.67
MOTA	747	N	ASP			30.899	63.611	86.662		16.37
ATOM	748	CA	ASP	A	108	31.305	65.002	86.772	1.00	17.18
ATOM	749	С	ASP			31.877	65.572	85.485	1.00	18.56
ATOM	750	0	ASP			31.324	66.472	84.877	1.00	18.77
ATOM	751	CB	ASP	A	108	32.282	65.144	87.947	1.00	16.92
ATOM	752	CG	ASP	A	108	32.862	66.530	88.143	1.00	21.34
ATOM	753	OD1	ASP	A	108	32,247	67.528	87.812		23.69
ATOM	754		ASP			33.983	66.642	88.614		27.32
ATOM	755	N	LEU			33.049	65.065	85.107		18.48
ATOM	756	CA	LEU			33.814	65.703	84.005		20.77
·	. • •					20.013	33.703	04.000	4.00	-V. //

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MOTA	757	С	LEU			32.954	65.922	82.752	1.00	21.46
ATOM	758	0	LEU	A	109	32.905	67.026	82.199	1.00	23.24
ATOM	759	CB	LEU	A	109	35.024	64.826	83.679	1.00	20.60
ATOM	760	CG	LEU	A	109	36.393	65.285	84.113		21.65
ATOM	761	CD1	LEU	A	109	37.408	64.213	84.096	1.00	17.20
MOTA	762	CD2	LEU	A	109	36.469	66.186	85.254	1.00	20.25
MOTA	763	N	ALA	A	110	32.206	64.812	82.422		21.81
MOTA	764	CA	ALA	A	110	31.336	64.771	81.215	1.00	20.79
ATOM	765	С	ALA	A	110	30.074	65.608	81.232	1.00	23.23
MOTA	766	0	ALA	A	110	29.504	65.885	80.182	1.00	23.66
MOTA	767	CB	ALA	A	110	30.921	63.333	80.868	1.00	20.49
ATOM	768	N	THR	A	111	29.672	66.038	82.465	1.00	20.15
ATOM	769	CA	THR	A	111	28.453	66.863	82.653	1.00	19.21
ATOM	770	С	THR			28.654	68.292	83.132	1.00	18.58
ATOM	771	0	THR	A	1.11	27.754	69.106	83.131	1.00	18.77
ATOM	772	CB	THR	A	111	27.469	66.257	83.628	1.00	18.46
MOTA	773	OG1	THR	A	111	28.011	66.399	84.949	1.00	23.25
ATOM	774	CG2	THR	A	111	27.094	64.802	83.347	1.00	15.78
ATOM	775	N	ARG	A	112	29.870	68.595	83.547	1.00	20.94
ATOM	776	CA	ARG	A	112	30.068	69.805	84.369	1.00	22.62
MOTA	777	C	ARG .	A	112	29.745	71.185	83.786	1.00	23.68
ATOM	778	0	ARG	A	112	29.035	72.025	84.325	1.00	21.09
ATOM	779	CB	ARG .	A	112	31.512	69.782	84.911	1.00	22.88
ATOM	780	CG	ARG .	A	112	31.847	70.852	85.952		22.67
ATOM	781	CD	ARG .	A	112	33.319	70.922	86.319	1.00	18.55
ATOM	782	NE	ARG .	A	112	33.831	69.709	86.930	1.00	22.11
ATOM	783	CZ	ARG .	A	112	35.138	69.496	86.853	1.00	21.99
ATOM	784	NH1	ARG .	A	112	35.949	70.322	86.227	1.00	23.29
ATOM	785	NH2	ARG .	A	112	35.623	68.436	87.414	1.00	23.83
ATOM	786	N	SER .	A	113	30.323	71.398	82.583		21.66
ATOM	787	CA	SER .	A	113	30.146	72.736	81.981		19.14
ATOM	788	C	SER .	A	113	28.721	73.124	81.629		20.09
ATOM	789	0	SER	A	113	28.288	74.263	81.806		23.06
ATOM	790	CB	SER .	A	113	31.029	72.919	80.732		24.64
MOTA	791	OG	SER :	A	113	30.812	71.854	79.778		25.18
ATOM	792	N	ALA :	A	114	27.955	72.094	81.186	1.00	
ATOM	793	ÇA	ALA :	A	114	26.510	72.272	80.944	1.00	17.15
ATOM	794	C	ALA	A	114	25.695	72.377	82.247		16.95
ATOM	795	0	ALA	A	114	24.890	73.293	82.402		17,79
ATOM	796	CB	ALA J	A	114	25.935	71.096	80.117		15.05
ATOM	797	N	LYS 2	A	115	25.993	71.462	83.200		
ATOM	798	CA	LYS 2			25.431	71.618	84.559		20.52
ATOM	799	С	LYS I	A	115	25.524	73.029	85.143		18.24
ATOM	800	0	LYS 2	A	115	24.535	73.710	85.429		19.61
ATOM	801	СВ	LYS 2			26.048	70.606	85.508		17.41
ATOM	802	CG	LYS A			25.304	69.294	85.482		22.93
ATOM	803	CD	LYS 2			25.867	68.477	86.654		26.73
ATOM	804	CE	LYS 2			25.353	67.039	86.850		26.14
ATOM	805	NZ	LYS Z			23.888	67.023	87.009		26.36
ATOM	806	N	ASP A			26.784	73.454	85.203		19.25
ATOM	807	CA	ASP 2			27.073	74.739	85.832	1.00	
ATOM	808	C	ASP A			26.589	75.938	85.076		23.51
ATOM	809	0	ASP Z			26.208	76.967	85.612		24.69
ATOM	810	CB	ASP 2			28.573	74.896	86.053		22,26
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MOTA	811	CG			116	29.203	73.871	87.029	1.00	25.80
MOTA	812		ASP			28.502	73.085	87.657	1.00	27.77
ATOM	813	OD2			116	30.431	73.847	87.166	1.00	28.44
ATOM	814	N			117	26.596	75.793	83.741		23.53
ATOM	815	CA			117	26.022	76.894	82.957	1.00	21.71
ATOM	816	С	HIS	A	117	24.496	77.044	83.026		20.75
ATOM	817	0	HIS	A	117	23.932	78.108	83.223	1.00	20.51
MOTA	818	CB	HIS	A	117	26.536	76.787	81.501	1.00	22.93
ATOM	819	CG	HIS	A	117	25.987	77.909	80.635	1.00	22.08
ATOM	820	ND1	HIS	A	117	26.531	79.128	80.459	1.00	23.33
ATOM	821	CD2	HIS	A	117	24.824	77.852	79.881	1.00	22.30
ATOM	822	CE1	HIS	A	117	25.741	79.836	79.615	1.00	23.49
ATOM	823	NE2	HIS	A	117	24.693	79.041	79.262	1.00	23.57
ATOM	824	N	TYR	A	118	23.825	75.906	82.825	1.00	19.95
ATOM	825	CA	TYR	A	118	22.363	76.013	82.795	1.00	19.91
ATOM	826	C	TYR	A	118	21.711	76.075	84.159	1.00	22.89
ATOM	827	0	TYR	A	118	20.615	76.596	84.278	1.00	22.53
ATOM	828	CB	TYR	A	118	21.702	74.869	82.020	1.00	18.70
MOTA	829	CG	TYR	A	118	22.112	74.964	80.550	1.00	19.86
ATOM	830	CD1	TYR	A	118	21.604	76.030	79.775	1.00	20.81
ATOM	831	CD2	TYR	A	118	22.998	74.004	80.005	1.00	20.19
ATOM	832	CE1	TYR	A	118	21.964	76.104	78.410	1.00	24.18
ATOM	833	CE2	TYR	Α	118	23.393	74.097	78.652		22.07
MOTA	834	CZ	TYR	A	118	22.841	75.133	77.869	1.00	24.85
MOTA	835	OH	TYR	A	118	23.138	75.231	76.525	1.00	25.02
MOTA	836	N	MET			22.385	75.453	85.158		22.72
ATOM	837	CA	MET	A	119	21.795	75.408	86.516		25.49
MOTA	838	С	MET	A	119	20.328	75.033	86.619		22.79
ATOM	839	0	MET			19.526	75.639	87.309		23.65
MOTA	840	CB	MET			22.009	76.758	87.200		31.90
ATOM	841	CG	MET			23.479	77.200	87.296		41.79
ATOM	842	SD	MET			23.683	78.779	88.163		50.25
ATOM	843	CE	MET			22.932	79.838	86.910		48.37
ATOM	844	N	ARG			19.958	74.021	85.840		21.05
ATOM	845	CA	ARG			18.529	73.782	85.704		19.45
ATOM	846	С	ARG			17.877	73.247	86.989		16.34
ATOM	847	ō	ARG			18.483	72.369	87.587		17.21
ATOM	848	CB	ARG			18.345	72.757	84.558		16.25
ATOM	849	CG	ARG			16.913	72.517	84.063		17.39
ATOM	850	CD	ARG			16.775	71.558	82.842		19.83
ATOM	851	NE	ARG			15.450	71.636	82.189		20.39
ATOM	852	CZ	ARG			14.929	70.642	81.479		12.14
ATOM	853		ARG			15.600	69.574	81.259		12.74
ATOM	854	NH2				13.724	70.767	81.007		15.75
ATOM	855	N	ILE			16.676	73.723	87.290		16.46
MOTA	856	CA	ILE			15.807	73.186	88.360		22.91
ATOM	857	C	ILE			15.258	71.795	88.080		23.50
MOTA	858	ō	ILE			14.648	71.733	87.043		26.14
ATOM	859	СВ	ILE			14.594	74.134	88.686		25.98
ATOM	860	CG1				14.986	75.592	88.783		29.16
ATOM	861	CG2				13.793	73.332	89.982		23.20
ATOM	862	CD1	ILE			16.075	75.914	89.785		29.73
ATOM	863	N	ARG			15.463	70.877	89.036		
ATOM	864	CA	ARG			14.883	69.534	88.976		21.01
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ATOM	865	С	ARG	A	122	13.372	69.532	89.091	1.00	17.61
ATOM	866	0	ARG	A	122	12.805	70.488	89.613		18.79
ATOM	867	CB	ARG	A	122	15.533	68.653	90.033		17.47
ATOM	868	CG	ARG	A	122	17.023	68.706	89.841		18.78
MOTA	869	ÇD	ARG	A	122	17.721	67.861	90.852		21.00
ATOM	870	NE			122	19.141	68.121	90.748		28.80
ATOM	871	CZ			122	19.914	67.081	90.491		34.31
ATOM	872	NH1			122	19.401	65.871	90.426		40.28
ATOM	873	NH2			122	21.196	67,257	90.289		32.47
ATOM	874	N			123	12.700	68.460	88.577		17.64
ATOM	875	CA			123	11,243	68.461	88.684		18.56
ATOM	876	C			123	10.668	68.630	90.118		20.86
ATOM	877	Õ			123	9.881	69.536	90.331		20.65
ATOM	878	СВ			123	10.808		88.004		
ATOM	879	CG			123	12.028	67.150			16.86
ATOM	880	CD			123	13.235	66.572	87.296		15.55
ATOM							67.291	87.881		15.48
	881	N			124	11.077	67.771	91.105		21.82
ATOM	882	CA	PHE			10.489	67.948	92.468		20.01
ATOM	883	C	PHE			10.541	69.394	93.016		16.73
ATOM	884	0	PHE			9.581	69.970	93.486		17.82
ATOM	885	CB	PHE			11.044	66.869	93.422		17.84
ATOM	886	CG	PHE			12.484	67.199	93.795		19.87
ATOM	887	CD1	PHE			12.748	68.117	94.850		20.96
ATOM	888		PHE			13.554	66.632	93.075		19.87
ATOM	889		PHE			14.068	68.524	95.134		21.78
MOTA	890	CE2				14.881	67.014	93.381		21.98
MOTA	891	CZ	PHE			15.129	67.975	94.386		23.27
ATOM	892	N	ALA			11.681	70.039	92.775	1.00	18.30
ATOM	893	CA	ALA			11.866	71.464	93.089		20.06
ATOM	894	С	ALA			11.033	72.481	92.291	1.00	24.90
MOTA	895	0	ALA	A	125	10.455	73.445	92.789	1.00	24.77
ATOM	896	CB	ALA			13.358	71.840	92.990	1.00	16.96
ATOM	897	N	PHE	A	126	10.941	72.202	90.977	1.00	23.91
ATOM	898	CA	PHE	A	126	10.017	72.958	90.145	1.00	22.66
MOTA	899	С	PHE	A	126	8.590	72.919	90.692	1.00	20.74
ATOM	900	0	PHE	A	126	7.910	73.945	90.785	1.00	21.66
ATOM	901	СВ	PHE	A	126	10.051	72.379	88.705		19.61
ATOM	902	CG	PHE	A	126	9.147	73.140	87.765		16.99
ATOM	903	CD1	PHE	A	126	9.669	74.211	87.022		15.15
ATOM	904	CD2	PHE	A	126	7.794	72.757	87.656		17.75
ATOM	905	CE1	PHE	A	126	8.824	74.913	86.144		14.16
ATOM	906	CE2	PHE	A	126	6.940	73.472	86.799		17.56
ATOM	907	CZ	PHE			7.471	74.538	86.048	1.00	
ATOM	908	N	TYR			8.183	71.664	91.002	1.00	
ATOM	909	CA	TYR			6.843	71.414	91.525	1.00	
ATOM	910	C	TYR			6.642	71.689	93.032	1.00	
ATOM	911	o	TYR			5.525				
ATOM	912	СB	TYR			6.370	69.994	91.207	1.00	
ATOM	913	CG	TYR			6.198	69.850	89.697	1.00	
ATOM	914		TYR			7.200	69.185	88.947	1.00	
ATOM	915		TYR			5.064	70.407	89.065		
ATOM	916	CE1				7.057	69.037		1.00	
ATOM	917	CE2						87.552	1.00	
ATOM	918	CZ	TYR			4.907	70.261	87.663	1.00	
221 (171	310	CA	TIK	u	12/	5.891	69.543	86.936	1.00	28.76

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ATOM	919	OH	TYR	A	127	5.716	69.314	85.588	1.00	27.53
ATOM	920	N	GLY	A	128	7.764	71.960	93.723	1.00	23.74
ATOM	921	CA	GLY	A	128	7.675	72.217	95.170	1.00	24.88
MOTA	922	С	GLY	A	128	7.138	71.011	95.924	1.00	24.57
ATOM	923	0	GLY	A	128	6.383	71.095	96.866	1.00	29.82
ATOM	924	N	VAL	A	129	7.527	69.854	95.426	1.00	24.16
ATOM	925	CA	VAL	A	129	7.144	68.604	96.044	1.00	23.21
ATOM	926	С	VAL	A	129	8.377	67.802	96.358	1.00	24.99
ATOM	927	0	VAL	A	129	9.529	68.188	96.189	1.00	25.90
ATOM	928	CB	VAL	A	129	6.193	67.776	95.187	1.00	22.38
ATOM	929	CG1	VAL	A	129	6.738	67.390	93.815	1.00	17.75
ATOM	930	CG2	VAL	A	129	4.895	68.549	95.125	1.00	24.52
ATOM	931	N	SER	A	130	8.089	66.617	96.832	1.00	25.98
ATOM	932	CA			130		65.724	96.973	1.00	29.71
ATOM	933	С	SER	A	130	9.322	64.672	95.895	1.00	29.73
MOTA	934	0	SER	A	130		64.485	95.103	1.00	30.28
ATOM	935	CB	SER	A	130	9.183	64.999	98.309		35.36
MOTA	936	OG	SER	A	130		64.244	98.427	1.00	41.61
MOTA	937	N	THR	A	131	10.440	63,952	95.934	1.00	30.35
MOTA	938	CA	THR	A	131	10.533	62.819	94.996	1.00	27.90
ATOM	939	C	THR	A	131	9.733	61.569	95.361	1.00	28.80
ATOM	940	0	THR	A	131	9.051	61.508	96.366	1.00	30.16
MOTA	941	CB	THR	A	131	11.996	62.471	94.783		26.62
ATOM	942	OG1	THR	A	131	12.500	61.841	95.953		29.21
ATOM	943	CG2			131	12.839	63.682	94.446		19.32
MOTA	944	N	CYS	A	132	9.835	60.528	94.551		27.12
ATOM	945	CA	CYS	A	132	9.203	59.271	94.996	1.00	27.75
ATOM	946	С	CYS	A	132	9.911	58.540	96.137		28.96
ATOM	947	0	CYS	A	132	9.556	57.487	96.634		28.80
ATOM	948	CB	CYS	A	132	9.081	58.274	93.831		24.68
MOTA	949	SG	CYS	A	132	10.538	57.273	93.459		24.12
MOTA	950	N	ASN	A	133	11.021	59.158	96.447	1.00	33.31
ATOM	951	CA	ASN	A	133	12.012	58.492	97.236		39.44
ATOM	952	С	ASN	A	133	12.008	58.776	98.750		45.19
MOTA	953	0	ASN	A	133	12.269	57.928	99.583		50.38
ATOM	954	CB	ASN	A	133	13.285	58.876	96.522		35.83
ATOM	955	CG	ASN	A	133	14.294	57.823	96.731		37.18
MOTA	956	OD1	ASN	A	133	15.478	58.059	96.663		38.83
MOTA	957	ND2	ASN	A	133	13.815	56.623	97.017	1.00	42.51
MOTA	958	N	THR	A	134	11.642	59.993	99.118	1.00	48.69
ATOM	959	CA	THR	A	134	12.585	61.110	98.925		56.17
MOTA	960	C	THR	A	134	13,935	61.171	99.735	1.00	61.49
ATOM	961	OCT1	THR	A	134	14.052	60.565	100.816		66.36
ATOM	962	OCT2	THR	A	134	14.937	61.779	99.291	1.00	63.18
ATOM	963	CB	THR	A	134	11.704	62.374	98.968		56.04
MOTA	964	OG1	THR	A	134	12.306	63.614	98.469		56.23
ATOM	965	CG2	THR	A	134	10.869		100.243		53.77
MOTA	966	N	GLN			16.953		100.819		00.00
ATOM	967	CA	GLN			17.845		102.027		99.78
ATOM	968	С	GLN			19.036		102.211		98.61
ATOM	969	0	GLN			19.386		103.324		97.54
MOTA	970	СВ	GLN			18.343		102.397		00.00
MOTA	971	CG	GLN			17.669		103.615		99.38
MOTA	972	CD	GLN			18.060		104.963		98.64

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ATOM	973	OE1	GLN	A	137	17.484	58.784	106.009	1.00 98.03
ATOM	974	NE2	GLN	A	137	19.086	59.847	104.972	1.00100.00
ATOM	975	N	ASP	A	138	19.627	61.949	101.081	1.00 97.91
ATOM	976	CA	ASP	A	138	20.479	63.151	101.162	1.00 95.99
ATOM	977	С	ASP	A	138	19.761	64.494	101.063	1.00 93.86
ATOM	978	0	ASP	Α	138	18.589	64.579	100.711	1.00 92.76
ATOM	979	CB	ASP	A	138	21.585	63.102	100.115	1.00 97.46
ATOM	980	CG	ASP	A	138	22.893	62.963	100.866	1.00100.00
ATOM	981	OD1	ASP	A	138	23.371	61.842	101.028	1.00100.00
MOTA	982	OD2	ASP	A	1.38	23.432	63.969	101.333	1.00100.00
ATOM	983	N	LYS	A	139	20.494	65.564	101.380	1.00 92.69
ATOM	984	CA	LYS	A	139	19.813	66.855	101,218	1.00 91.50
ATOM	985	C	LYS	A	139	19.719	67.313	99.775	1.00 88.92
MOTA	986	0	LYS	A	139	20.557	68.082	99.308	1.00 89.47
ATOM	987	CB	LYS	A	139	20.464	67.987	102.010	1.00 94.21
ATOM	988	CG	LYS	A	139	19.574	69.246	102.020	1.00 96.82
ATOM	989	CD	LYS	A	139	20.362	70.569	102.123	1.00 99.43
ATOM	990	CE	LYS	A	139	20.785	71.241	100.793	1.00100.00
ATOM	991	NZ	LYS	A	139	21.686	70.402	99.978	1.00100.00
ATOM	992	N			140	18.654	66.828	99.114	1.00 85.87
ATOM	993	CA	LEU	A	140	18.426	67.018	97.660	1.00 79.60
ATOM	994	С			140	18.772	68.392	97.043	1.00 75.62
ATOM	995	0	LEU	A	140	18.242	69.442	97.416	1.00 75.74
ATOM	996	CB	LEU	A	140	16.981	66.620	97.283	1.00 77.27
ATOM	997	CG			140	16.640	65.142	97.462	1.00 74.31
ATOM	998	CD1			140	17.569	64.263	96.645	1.00 73.21
ATOM	999	CD2			140	15.178	64.838	97.167	1.00 73.91
ATOM	1000	N			141	19.713	68.357	96.067	1.00 70.08
ATOM	1001	CA			141	19.868	69.619	95.320	1.00 64.86
ATOM	1002	C			141	18.713	69.941	94.396	1.00 61.18
ATOM	1003	0			141	18.189	69.080	93.708	1.00 61.77
ATOM	1004	CB			141	21.127	69.682	94.461	1.00 64.49
ATOM	1005	OG			141	21.354	71.028	94.002	1.00 66.43
ATOM	1006	N	LYS			18.349	71.235	94.379	1.00 57.81
ATOM	1007	CA	LYS			17.238	71.661	93.493	1.00 54.35
ATOM	1008	С	LYS			17.498	71.829	91.964	1.00 45.94
ATOM	1009	0	LY5			16.615	72.091	91.202	1.00 40.89
ATOM	1010	СВ	LYS			16.631	72.953	94.061	1.00 57.69
ATOM	1011	CG	LYS			17.518	74.222	93.959	1.00 62.63
ATOM	1012	CD	LYS			16.625	75.420	93.545	1.00 68.07
ATOM	1013	CE	LYS			17.200	76.856	93.475	1.00 71.28
ATOM	1014	NZ	LYS			16.136	77.844	93.162	1.00 70.88
ATOM	1015	N	ASN			18.772	71.719	91.611	1.00 45.99
ATOM	1016	CA	ASN			19.527	72.392	90.538	1.00 44.71
ATOM	1017	C	ASN			20.592	71.481	89.878	1.00 42.57
ATOM	1018	0	ASN			20.794	70.322	90.283	1.00 40.17
ATOM	1019	CB	ASN			20.343	73.547	91.124	1.00 50.34
ATOM ATOM	1020 1021	CG	ASN ASN			19.624	74.818	90.876	1.00 55.19
ATOM	1021		ASN			18.441	74.944	91.098	1.00 59.21
ATOM	1022	NDZ N				20.366	75.786	90.389	1.00 59.88
ATOM	1023	CA	GLY			21.265	72.084	88.819	1.00 39.72
ATOM	1024	CA	GLY			22.264	71.401	87.958	1.00 26.74
ATOM	1025		GLY			21.691	70.105	87.407	1.00 21.44
ALUM	TOSO	0	GLY	A	1.44	22.343	69.071	87.322	1.00 25.78

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ATOM	1027	N	SER	A	145	20.380	70.181	87.105	1.00	19.15
ATOM	1028	ÇA	SER	A	145	19.590	69.023	86.596	1.00	20.14
ATOM	1029	С	SER	A	145	20.077	68.461	85.223	1.00	20.58
ATOM	1030	0	SER	A	145	20.121	67.279	84.943	1.00	21.22
ATOM	1031	CB	SER	A	145	18.171	69.499	86.338	1.00	18.89
ATOM	1032	OG	SER	A	145	17.219	68.526	86.712		28.02
ATOM	1033	N	TYR	A	146	20.531	69.419	84.399		22.70
ATOM	1034	CA			146	20.867	69.170	82.968		19.51
ATOM	1035	С			146	22.366	69.260	82.633		16.00
ATOM	1036	0			146	22.930	70.339	82.736		17.85
ATOM	1037	СВ			146	20.097	70.216	82.114		18.79
ATOM	1038	CG			146	20.211	69.933	80.600		20.29
ATOM	1039	CD1			146	19.310	69.027	80.015		19.58
ATOM	1040	CD2			146	21.205	70.582	79.824		20.42
ATOM	1041	CE1			146	19.389	68.781	78.631		23.13
ATOM	1042	CE2			146	21.287	70.337	78.424		21.68
ATOM	1043	CZ			146	20.349	69.449	77.849		20.03
ATOM	1044	ОН			146	20.320	69.236	76.483		21.40
ATOM	1045	N			147	22.994	68.144	82.187		16.52
ATOM	1046	CA			147	22.385	66.793	82.188		17.87
ATOM	1047	C			147	22.496	66.069	83.580		20.67
ATOM	1048	ō			147	23.198	66.531	84.480		22.35
ATOM	1049	СВ			147	23.250	66.107	81.122	1.00	
ATOM	1050	CG			147	24.649	66.716	81.297		15.06
ATOM	1051	CD			147	24.356	68.168	81.630		16.28
ATOM	1052	N			148	21.827	64.908	83.662		18.73
ATOM	1052	CA			148	21.951	64.019	84.823		19.51
ATOM	1054	C			148	23.269				
ATOM	1055	o			148	23.601	63.281	84.930		19.93
ATOM	1056	СВ			148	20.828	62.439	84.108		20.09
ATOM	1057	OG			148		62.997	84.807		18.55
ATOM	1057	И			149	20.990	61.966	85.780		19.29
ATOM	1059	CA				24.036	63.618	85.972		18.26
ATOM	1060	C			149 149	25.284	62.888	86.231		16.00
ATOM						25.096	61.411	86.577		19.38
	1061	0			149	25.791	60.549	86.044		21.04
ATOM	1062	N			150	24.085	61.096	87.427		20.25
ATOM	1063	CA			150	23.690	59.680	87.624		19.01
MOTA	1064	C	HIS			23.381	58.861	86.330		19.93
ATOM	1065	0	HIS			23.833	57.725	86.145		20.78
ATOM	1066	CB			150	22.507	59.533	88.619		18.01
ATOM	1067	CG			150	22.162	58.068	88.909		20.60
MOTA	1068	ND1	HIS			22.864	57.235	89.730		23.41
MOTA	1069		HIS			21.117	57.313	88.374		21.91
ATOM	1070	CE1				22.287	55.996	89.732		22.83
ATOM	1071		HIS			21.220	56.051	88.893		24.19
ATOM	1072	N	THR			22.593	59.482	85.432		18.98
ATOM	1073	CA	THR			22.325	58.814	84.132		17.17
MOTA	1074	C	THR			23.548	58.652	83.228		13.61
ATOM	1075	0	THR			23.814	57.594	82.659		17.00
ATOM	1076	CB	THR			21.270	59.590	83.407		16.57
ATOM	1077	OG1				20.137	59.738	84.258		18.00
MOTA	1078	CG2	THR			20.898	58.983	82.045	1.00	13.22
ATOM	1079	N	SER			24.361	59.722	83.197	1.00	14.30
ATOM	1080	CA	SER	A	152	25.687	59.598	82.557	1.00	15.99

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MOTA	1081	C	SER	A	152	26.575	58.446	83.029	1.00	19.72
ATOM	1082	0	SER	A	152	27.086	57.628	82.255	1.00	17.38
ATOM	1083	CB	SER	A	152	26.434	60.927	82.644	1.00	
MOTA	1084	OG	SER	A	152	27.648	60.861	81.911	1.00	16.08
ATOM	1085	N	ILE	A	153	26.662	58.340	84.403	1.00	20.60
ATOM	1086	CA	ILE	A	153	27.272	57.121	85.017	1.00	16.15
MOTA	1087	С	ILE	A	153	26.622	55.802	84.631		10.76
ATOM	1088	O	ILE	A	153	27.293	54.850	84.262		14.38
ATOM	1089	CB	ILE	A	153	27.384	57.170	86.608		15.02
MOTA	1090	CG1	ILE	A	153	28.187	58.421	86.963		16.37
ATOM	1091	CG2	: ILE	A	153	28.154	55.944	87.164		12.98
ATOM	1092	CD1	ILE	A	153	27.870	59.034	88.338		16.58
ATOM	1093	N	GLY	A	154	25.285	55.763	84.720	1.00	
ATOM	1094	CA	GLY	A	154	24.662	54.476	84.397	1.00	13.63
ATOM	1095	C	GLY	A	154	24.843	54.033	82.910		16.00
ATOM	1096	0	GLY	A	154	25.022	52.866	82.571	1.00	16.37
ATOM	1097	N	TRP	A	1.55	24.801	55.047	82.025		16.77
ATOM	1098	CA	TRP	A	155	24.960	54.747	80.589		16.73
ATOM	1099	С	TRP	A	155	26.378	54.351	80.200		15.90
MOTA	1100	0	TRP	A	155	26.656	53.301	79.628		18.41
ATOM	1101	CB	TRP	A	155	24.442	55.940	79.771		16.52
ATOM	1102	CG	TRP	A	155	24.320	55.475	78.321		19.11
ATOM	1103	CD1	TRP	A	155	25.009	56.015	77.210		18.94
ATOM	1104	CD2	TRP	A	155	23.471	54.426	77.765		18.98
MOTA	1105	NE1	TRP	A	155	24.679	55.352	76.044		17.77
ATOM	1106	CE2	TRP	A	155	23.781	54.322	76.357	1.00	22.22
MOTA	1107	CE3	TRP	A	155	22.585	53.486	78.337	1.00	19.43
ATOM	1108	CZ2	TRP	A	155	23.084	53.377	75.564		17.99
ATOM	1109	CZ3	TRP	A	155	21.913	52.538	77.537		19.46
ATOM	1110	CH2	TRP	A	155	22.191	52.464	76.158	1.00	17.84
ATOM	1111	N	ALA	A	156	27.299	55.209	80.623		15.37
ATOM	1112	CA	ALA			28.702	54.836	80.515		14.36
ATOM	1113	С	ALA	A	156	29.156	53.503	81.108		19.46
ATOM	1114	0	ALA	A	156	29.895	52.723	80.528	1.00	19.86
ATOM	1115	CB	ALA	A	156	29.564	55.918	81.136		15.83
ATOM	1116	N	THR	A	157	28.651	53.207	82.327		19.95
ATOM	1117	CA	THR	A	157	28.820	51.832	82.831		17.52
ATOM	1118	С	THR	A	157	28.177	50.744	81.994		15.22
ATOM	1119	0	THR	A	157	28.825	49.745	81.765		19.09
ATOM	1120	CB	THR	A	157	28.328	51.667	84.291		14.92
ATOM	1121	OG1				28.932	52.679	85.054	1.00	18.29
ATOM	1122	CG2	THR	A	157	28.620	50.327	84.944	1.00	13.21
ATOM	1123	N	ALA			26.930	50.947	81.535	1.00	14.63
ATOM	1124	CA	ALA	A	158	26.365	49.936	80.621		17.10
MOTA	1125	С	ALA			27.213	49.686	79.354		15.52
ATOM	1126	0	ALA	A	158	27.539	48.565	79.025		16.52
ATOM	1127	CB	ALA	A	158	24.942	50.300	80.203	1.00	13.99
ATOM	1128	N	LEU			27.655	50.766	78.705		17.91
ATOM	1129	CA	TEA	A	159	28.613	50.615	77.580		17.69
MOTA	1130	С	LEU			29.895	49.851	77.846		19.93
ATOM	1131	0	LEU			30.277	48.954	77.092		19.21
ATOM	1132	CB	LEU			28,959	51.971	76.939		14.08
ATOM	1133	CG	LEU			27.744	52.759	76.396		11.97
MOTA	1134	CD1	LEU	A .	159	27.045	52.105	75.210		12.90

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ATOM	1135	CD2	LEU	A	159	28.177	54.158	76.046	1.00	12.40
ATOM	1136	N	VAL	A	160	30.547	50.181	79.014		21.70
ATOM	1137	CA	VAL	A	160	31.713	49.315	79.310	1.00	20.85
MOTA	1138	С	VAL	A	160	31.429	47.877	79.767	1.00	21.33
ATOM	1139	0	VAL	A	160	32.086	46.918	79.409	1.00	20.74
ATOM	1140	CB	VAL	A	160	32.574	50.032	80.367	1.00	21.59
ATOM	1141	CG1	VAL	A	160	33.518	49.143	81.174	1.00	20.11
ATOM	1142	CG2	VAL	A	160	33.299	51.307	79.977	1.00	20.10
ATOM	1143	N	LEU	A	161	30.351	47.711	80.542	1.00	21.86
ATOM	1144	CA	LEU	A	161	29.850	46.366	80.838	1.00	20.50
ATOM	1145	С	LEU	A	161	29.462	45.490	79.613	1.00	23.16
ATOM	1146	О	LEU	A	161	29.753	44.294	79.564	1.00	22.89
ATOM	1147	CB	LEU	A	161	28.623	46.472	81.733	1.00	18.65
ATOM	1148	CG	LEU	A	161	28.685	46.438	83.262	1.00	20.99
MOTA	1149	CD1	LEU	A	161	27.473	46.378	84.194	1.00	21.43
ATOM	1150	CD2	LEU	A	161	29.729	45.492	83.855	1.00	19.30
ATOM	1151	N	ALA	A	162	28.755	46.146	78.648	1.00	21.20
ATOM	1152	CA	ALA	A	162	28.384	45.496	77.361	1.00	19.14
ATOM	1153	C	ALA	A	162	29.591	44.948	76.586	1.00	17.15
ATOM	1154	0	ALA	A	162	29.620	43.812	76.133	1.00	20.85
ATOM	1155	CB	ALA	A	162	27.581	46.464	76.500	1.00	17.38
ATOM	1156	N	GLU	A	163	30.663	45.745	76.603	1.00	14.74
ATOM	1157	CA	GLU	A	163	31.962	45.262	76.118	1.00	17.48
MOTA	1158	С	GLU	A	163	32.648	44.080	76.824	1.00	22.07
ATOM	1159	0	GLU	A	163	33.271	43.216	76.227	1.00	23.96
ATOM	1160	СВ	GLU	A	163	32.915	46.448	76.000	1.00	13.52
ATOM	1161	CG	GLU	A	163	34.227	46.018	75.359	1.00	13.04
ATOM	1162	CD	GLU	A	163	35.240	47.119	75.338	1.00	15.88
ATOM	1163	OE1	GLU	A	163	36.427	46.814	75.269	1.00	19.78
ATOM	1164	QE2	GLU	A	163	34.873	48.290	75.377	1.00	20.10
ATOM	1165	N	ILE	A	164	32.504	44.039	78.153	1.00	20.17
ATOM	1166	CA	ILE	A	164	32.996	42.869	78.905	1.00	18.72
ATOM	1167	С	ILE	A	164	32.164	41.609	78.757	1.00	17.39
ATOM	1168	0	ILE	A	164	32.635	40.481	78.674	1.00	20.44
ATOM	1169	СВ	ILE	A	164	33.132	43,293	80.382	1.00	20.67
ATOM	1170	CG1	ILE	A	164	34.222	44.361	80.452	1.00	18.83
ATOM	1171	CG2	ILE	A	164	33.398	42.110	81.345	1.00	20.70
ATOM	1172	CD1	ILE	А	164	34.144	45.084	81.793	1.00	21.31
ATOM	1173	N	ASN	A	165	30.869	41.846	78.704	1.00	18.41
ATOM	1174	CA	ASN	A	165	29.979	40.712	78.524	1.00	21.36
ATOM	1175	С	ASN	A	165	28.957	40.867	77.375	1.00	23.74
ATOM	1176	0	ASN	A	165	27.753	40.988	77.563	1.00	23.14
ATOM	1177	CB	ASN	A	165	29.324	40.404	79.878	1.00	21.69
ATOM	1178	CG	ASN	A	165	28.471	39.156	79.861	1.00	25.72
ATOM	1179	ODI	ASN	A	165	28.469	38.307	78.967	1.00	29.37
MOTA	1180	ND2	ASN	A	165	27.730	39.051	80.951	1.00	27.39
ATOM	1181	N	PRO	A	166	29.466	40.814	76.118	1.00	25.93
ATOM	1182	CA	PRO	A	166	28.556	40.971	74.957	1.00	26.27
MOTA	1183	C	PRO			27.447	39.924	74.837		24.06
ATOM	1184	0	PRO			26.361	40.184	74.360		24.11
ATOM	1185	CB	PRO			29.517	41.040	73.781		25.15
MOTA	1186	CG	PRO			30.731	40.254	74.255		27.68
ATOM	1187	CD	PRO			30.849	40.613	75.728		24.89
ATOM	1188	N	GLN			27.679	38.741	75.385		25.15
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ATOM	1189	CA			167	26.552	37.804	75.508	1.0	27.09
ATOM	1190	С	GLN	A	167	25.275	38.321	76.189	1.0	
ATOM	1191	0	GLN	A	167	24.162	37.932	75.883	1.00	
ATOM	1192		GLN	A	167	27.080		76.282	1.00	
ATOM	1193	CG	GLN	A	167	27.386	35.380	75.468	1.00	
ATOM	1194	CD	GLN	A	167	26.074	34.747	75.000	1.00	
ATOM	1195	OE:	l GLN	A	167	25.950	34.245	73.889	1.00	
ATOM	1196	NE:	2 GLN	A	167	25.047	34.780	75.867	1.00	
ATOM	1197	N	ARG	A	168	25.513	39.242	77.156		26.04
ATOM	1198	CA	ARG	A	168	24.389	39.900	77.837		25.63
ATOM	1199	C	ARG	A	168	24.242	41.368	77.558		23.88
ATOM	1200	0	ARG	A	168	23.632	42.106	78.308	1.00	
ATOM	1201	CB	ARG	A	168	24.452	39.638	79.337	1.00	
ATOM	1202	CG	ARG	A	168	24.087	38.171	79.408	1.00	
ATOM	1203	CD	ARG	A	168	23.986	37.617	80.802		33.72
ATOM	1204	NE	ARG	A	168	22.970	38.302	81.587		35.38
ATOM	1205	CZ	ARG	A	168	21.729	37.877	81.680		35.91
ATOM	1206		. ARG			20.908	38.482	82.490		34.99
ATOM	1207	NH2	ARG	A	168	21.314	36.867	80.975		39.28
ATOM	1208	N	GLN	A	169	24.838	41.810	76.424		23.22
MOTA	1209	CA	GLN	A	169	24.820	43.228	76.080		19.99
MOTA	1210	С	GLN	A	169	23.441	43.850	76.067		21.38
ATOM	1211	0	GLN	A	169	23.216	44.969	76.492		24.60
ATOM	1212	CB	GLN	A	169	25.571	43.510	74.769		22.07
MOTA	1213	CG	GLN	A	169	24.970	42.878	73.494		20.93
ATOM	1214	CD	GLN .	A	169	25.716	43.287	72.207		23.42
MOTA	1215	OE1	GLN .	A	169	26.680	44.055	72.202		24.82
ATOM	1216	NE2	GLN .	A	169	25.186	42.738	71.109		13.86
ATOM	1217	N	ASN .	A	170	22.455	43.089	75.600		20.48
ATOM	1218	CA	ASN .			21.138	43.736	75.525		20.97
ATOM	1219	С	ASN .			20.484	44.007	76.892		19.73
ATOM	1220	0	ASN .	A	170	19.852	45.020	77.128		18.90
ATOM	1221	CB	ASN A	A	170	20.195	42.930	74.595		24.43
ATOM	1222	CG	ASN A	A	170	20.763	42.881	73.153	1.00	25.82
ATOM	1223	OD1				20.842	43.862	72.440	1.00	26.22
ATOM	1224	ND2	ASN 2	A.	170	21.197	41.709	72.734	1.00	25.47
ATOM	1225	N	GLU 2	A.	171	20.680	43.042	77.790		21.27
ATOM	1226	CA	GLU 2			20.166	43.146	79.157	1.00	19.84
ATOM	1227	C	GLU A			20.849	44.220	79.926	1.00	16.23
ATOM	1228	0	GLU A			20.199	45.026	80.575	1.00	20.87
ATOM	1229	CB	GLU 2			20.317	41.822	79.892	1.00	20.47
ATOM	1230	CG	GLU A			19.412	40.750	79.312	1.00	24.15
ATOM	1231	CD	GLU A		171	20.157	39.825	78.376	1.00	26.37
ATOM	1232		GLU A		171	21.076	40.256	77.685		23.54
ATOM	1233		GLU A			19.801	38.645	78.363		32.14
ATOM	1234	N	ILE A			22.169	44.236	79.751	1.00	17.53
MOTA	1235	CA	ILE A			23.029	45.318	80.257	1.00	16.90
ATOM	1236	C	ILE A			22.679	46.733	79.813		20.21
ATOM	1237	0	ILE A			22.454	47.636	80.617		18.91
ATOM	1238	CB	ILE A			24.507	44.992	79.956	1.00	17.00
MOTA	1239		ILE A			25.000	43.685	80.613		15.78
ATOM	1240		ILE A			25.426	46.163	80.300		18.13
ATOM	1241		ILE A			26.426	43.320	80.163		13.83
ATOM	1242	N	LEU A	. 1	173	22.575	46.909	78.468		20.75

[図33]

ATOM	1243	CA			173	22.107	48.206	77.946	1.00	17.70
ATOM	1244	C	LEU	A	173	20.699	48.622	78.396	1.00	16.26
ATOM	1245	0	LEU	A	173	20.376	49.774	78.663	1.00	18.18
ATOM	1246	CB	LEU	A	173	22.176	48.201	76.419	1.00	16.81
ATOM	1247	CG	LEU	A	173	23.600	48.021	75.966	1.00	18.73
ATOM	1248	CD1	LEU	A	173	24.410	49.282	76.138	1.00	16.97
ATOM	1249	CD2	LEU	A	173	23.619	47.550	74.505		23.91
ATOM	1250	N			174	19.835	47.625	78.475		15.86
ATOM	1251	CA	LYS	A	174	18.494	47.970	78.945		19.21
ATOM	1252	С	LYS	A	174	18.453	48.415	80.429		21.86
ATOM	1253	0	LYS	A	174	17.800	49.404	80.737		20.00
ATOM	1254	CB			174	17.577	46.773	78.700		20.19
ATOM	1255	CG			174	16.094	47,130	78.731		27.84
ATOM	1256	CD			174	15.799	48.368	77.858		36.14
ATOM	1257	CE			174	14,309	48.628	77.774		40.56
ATOM	1258	NZ			174	13.775	48.622	79.156		49.45
ATOM	1259	N			175	19.250	47.698	81.286		21.93
ATOM	1260	CA			175	19.476	48.145	82.686		19.65
ATOM	1261	C			175	20.037	49.561	82.807		16.70
ATOM	1262	ō			175	19.476	50.419	83.468		18.12
ATOM	1263	СВ			175	20.345	47.126	83.467		21.25
ATOM	1264	CG			175	20.608	47.477	84.954		22.00
ATOM	1265	CD			175	19.259	47.669	85.656		25.63
ATOM	1266	NE			175	19.435	47.923	87.095		29.77
ATOM	1267	CZ			175	18.601	48.672	87.834		27.58
ATOM	1268	NH1			175	17.550	49.301	87.355		
MOTA	1269	NH2			175	18.792	48.780			24.74
ATOM	1270	N			176	21.122		89.073		27.45
ATOM	1271	CA			176		49.800	82.048		17.53
ATOM	1272	C			176	21.698	51.143 52.214	81.893		17.18
ATOM	1273	0			176	20.679		81.552		21.18
ATOM	1274	И				20.582	53.278	82.149		21.85
ATOM	1275	CA	TYR		177	19.847	51.887	80.547		22.48
ATOM	1275	C				18.756	52.787	80.117		20.51
ATOM					177	17.754	53.106	81.242		18.92
	1277	O	TYR			17.406	54.246	81.542		15.84
ATOM	1278	CB	TYR			18.007	52.120	78.939		23.76
ATOM	1279	CG CD1	TYR			17.210	53.131	78.177		24.47
ATOM	1280	CD1	TYR			15.817	53.306	78.397		26.61
ATOM	1281	CD2	TYR			17.941	53.869	77.236		29.40
ATOM	1282	CE1	TYR			15.139	54.308	77.661		29.08
ATOM	1283	CE2	TYR			17.270	54.855	76.515		30.70
ATOM	1284	CZ	TYR			15.899	55.092	76.747		32.01
MOTA	1285	ОН	TYR			15.401	56.167	76.020		41.52
ATOM	1286	N	GLU			17.354	52.008	81.867		19.45
ATOM	1287	CA	GLU			16.429	52.094	82.972		22.02
ATOM	1288	C	GLU	A	178	16.820	52.802	84.236		20.09
ATOM	1289	0	GLU			16.001	53.492	84.805		21.41
MOTA	1290	СВ	GLU			16.010	50.731	83.357		25.45
ATOM	1291	CG	GLU			15.173	50.032	82.303		34.73
ATOM	1292	CD	GLU			13.893	50.810	81.951		40.36
ATOM	1293	OE1				13.432	51.667	82.707		33.51
ATOM	1294	OE2	GLU			13.352	50.556	80.876		45.77
ATOM	1295	N	LEU			18.090	52.695	84.609	1.00	19.63
ATOM	1296	CA	LEU	A	179	18.655	53.567	85.665	1.00	19.10

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ATOM	1297			A 179	18.366	55.051	85.511	1.00 20.25
ATOM	1298			A 179	17.838	55.726	86.374	1.00 19.26
ATOM	1299			A 179	20.178	53.361	85.813	1.00 17.45
ATOM	1300			A 179	20.610		86.426	1.00 18.23
ATOM	1301	CD	-	A 179	20.093	51.829	87.873	
ATOM	1302	CD:	2 LEU.	A 179	22.124	51.811	86.340	1.00 16.34
ATOM	1303	N	GLY .	A 180	18.647	55.545	84.290	1.00 19.84
ATOM	1304	CA	GLY 2	A 180	18.171	56.903	83.987	1.00 15.90
ATOM	1305	C	GLY 2	A 180	16.655	57.072	84.027	1.00 17.05
ATOM	1306	0	GLY :	A 180	16.130	58.031	84.564	1.00 19.49
ATOM	1307	N	GLN 2	A 181	15.923	56.068	83.478	1.00 18.19
ATOM	1308	CA		A 181	14.448	56.207	83.522	1.00 17.92
ATOM	1309	C	GLN 2	A 181	13.822	56.265	84.938	1.00 18.20
ATOM	1310	0	GLN Z		12.903	57.008	85.258	1.00 19.66
ATOM	1311	CB		181	13.716	55.189	82.620	1.00 15.39
ATOM	1312	CG		181	13.936	55.324	81.073	1.00 15.18
ATOM	1313	CD		1 181	13.823	56.763	80.634	1.00 13.10
ATOM	1314	OE			14.769	57.400	80.176	1.00 17.23
ATOM	1315	NE2			12.626	57.300	80.812	
ATOM	1316	N	SER A		14.440	55.497		1.00 12.82
ATOM	1317	CA	SER A		14.156	55.600	85.821	1.00 20.03
ATOM	1318	c	SER A		14.209	56.973	87.273	1.00 16.56
ATOM	1319	ō	SER A		13.305		87.871	1.00 14.78
ATOM	1320	CB	SER A		15.056	57.369	88.582	1.00 19.49
ATOM	1321	OG	SER A		14.563	54.630	88.021	1.00 15.31
ATOM	1322	N	ARG A			53.332	87.714	1.00 16.70
ATOM	1323	CA	ARG A		15.246	57.738	87.523	1.00 16.99
ATOM	1324	C	ARG A		15.289	59.169	87.858	1.00 15.54
ATOM	1325	o	ARG A		14.161	60.085	87.309	1.00 19.55
ATOM	1326	СВ	ARG A		13.693	61.039	87.956	1.00 17.04
ATOM	1327	CG			16.661	59.785	87.569	1.00 14.08
ATOM	1328	CD	ARG A		17.811	59.024	88.265	1.00 19.49
ATOM	1329				17.716	59.007	89.816	1.00 21.46
ATOM	1330	NE	ARG A		18.519	57.930	90.434	1.00 22.16
ATOM		CZ	ARG A		19.509	58.121	91.300	1.00 19.15
	1331		ARG A		19.913	59.335	91.541	1.00 16.50
ATOM	1332		ARG A		20.042	57.081	91.914	1.00 18.34
ATOM	1333	N	VAL A		13.681	59.704	86.076	1.00 21.38
ATOM	1334	CA	VAL A		12.494	60.417	85.589	1.00 19.02
ATOM	1335	C	VAL A		11.194	60.051	86.301	1.00 16.98
ATOM	1336	0	VAL A		10.468	60.913	86.800	1.00 18.45
ATOM	1337	CB	VAL A		12.368	60.194	84.070	1.00 19.28
ATOM	1338		VAL A		11.057	60.717	83.486	1.00 17.84
ATOM	1339		VAL A		13.517	60.605	83.169	1.00 15.69
ATOM	1340	N	ILE A	185	10.977	58.734	86.408	1.00 17.32
ATOM	1341	CA	ILE A	185	9.834	58.241	87.202	1.00 21.14
ATOM	1342	C	ILE A	185	9.790	58.797	88.672	1.00 22.77
ATOM	1343	0	ILE A	185	8.749	59.230	89.142	1.00 22.95
ATOM	1344	CB	ILE A		9.810	56.695	87.193	1.00 20.65
ATOM	1345	CG1	ILE A	185	9.555	56.195	85.758	1.00 18.43
ATOM	1346	CG2	ILE A	185	8.776	56.133	88.202	1.00 18.29
ATOM	1347	CD1	ILE A	185	9.914	54.734	85.494	1.00 14.60
ATOM	1348	N	CYS A		10.976	58.837	89.332	1.00 20.34
ATOM	1349	CA	CYS A		11.005	59.262	90.745	1.00 20.34
MOTA	1350	С	CYS A		10.979	60.766	90.743	
	-				-0.013	JV. / UU	20.33I	1.00 22.80

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ATOM	1351	0	CYS	A	186	10.	775 61	.304	92.009	1.00	23.98
ATOM	1352	CB	CYS	A	186	12.3	220 58	.669	91.429	1.00	21.60
ATOM	1353	SG	CYS	A	186	12.	075 58	.576	93.237	1.00	25.16
ATOM	1354	N	GLY	A	187	11.	149 61	.494	89.814	1.00	20.34
ATOM	1355	CA	GLY	A	187	11.0	023 62	.949	89.981	1.00	13,75
MOTA	1356	C	GLY	Α	187	12.3	351 63	.676	90.197	1.00	14.55
ATOM	1357	0	GLY	A	187	12.4	136 64	.871	90.453	1.00	
ATOM	1358	N	TYR	A	188	13.4			90.072	1.00	
ATOM	1359	CA	TYR	A	188	14.7			90.226		16.85
ATOM	1360	C	TYR	A	188	15.2	214 64	.366	89.046		20.24
ATOM	1361	0	TYR	A	188	15.9	79 65	.318	89.192		18.42
ATOM	1362	CB	TYR	A	188	15.7			90.479		21.64
ATOM	1363	CG	TYR	A	188	15.6	83 61	.913	91.899	1.00	27.20
ATOM	1364	CD1	TYR	A	188	16.7	148 62		92.737		32.00
MOTA	1365	CD2	TYR	A	188	14.6	604 61	.128 !	92.365	1.00	30.81
MOTA	1366	CE1	TYR	A	188	16.7	727 61	.784 9	94.071	1.00	35.38
ATOM	1367	CE2	TYR	A	188	14.5	70 60	.695	93.705	1.00	30.65
MOTA	1368	CZ	TYR	A	188	15.6	24 61		94.556	1.00	35.88
ATOM	1369	HO	TYR	A	188	15.6	60 60	.787 5	95.912	1.00	41.39
ATOM	1370	N	HIS	A	189	14.7	51 63	.890	87.862		19.35
ATOM	1371	CA	HIS	A	189	15.2	15 64	.475	86.589		19.83
MOTA	1372	C	HIS	A	189	14.1	.08 64		85.551		16.85
ATOM	1373	0	HIS	A	189	13.2			35.533		18.10
ATOM	1374	CB	HIS	A	189	16.3	60 63		36.032		15,43
ATOM	1375	CG	HIS	A	189	17.6	77 64		36.589	1.00	
MOTA	1376	ND1			189	18.1	54 65		36.414	1.00	
MOTA	1377	CD2	HIS	A	189	18.5	81 63		37.382		17.58
ATOM	1378	CE1	HIS	A	189	19.3	47 65.	.503 8	37.080		16.38
ATOM	1379	NE2	HIS	A	189	19.5	87 64	.304 8	37.667	1.00	
ATOM	1380	N	TRP	A	190	14.1	83 65.	.611 8	34.723	1.00	18.99
ATOM	1381	CA	TRP	A	190	13.3	41 65.	.719 8	33.490	1.00	17.22
ATOM	1382	C	TRP	A	190	13.7	68 64.	. 690 8	32.453	1.00	15.79
ATOM	1383	0	TRP	A	190	14.9	42 64		32.416		15.77
ATOM	1384	CB	TRP	A	190	13.5	02 67	102 8	32.855		16.12
ATOM	1385	CG	TRP	A	190	13.1			33.857		12.39
ATOM	1386	CD1	TRP	A	190	14.0	33 69.	.073 E	4.454		10.88
MOTA	1387	CD2	TRP	A	190	11.8			4.295		14.35
ATOM	1388	NE1	TRP	A	190	13.3	43 69.		5.186		13.77
MOTA	1389	CE2	TRP	A	190	11.9		731 8	5.141		10.71
MOTA	1390	CE3	TRP	A	190	10.5	05 68.	036 8	4.094		14.68
ATOM	1391	CZ2	TRP	A	190	10.8	45 70.	401 8	5.666	1.00	12.89
ATOM	1392	CZ3	TRP	A	190	9.3	93 68.	700 8	4.672		16.93
ATOM	1393	CH2	TRP	A	190	9.5	57 69.		5.441		12.82
MOTA	1394	N	GLN	A	191	12.8	59 64.	188 8	1.613	1.00	16.92
ATOM	1395	CA	GLN	A	191	13.3	16 63.	234 8	0.569	1.00	17.49
ATOM	1396	C	GLN	A	191	14.5	19 63.	720 7	9.696	1.00	15.64
MOTA	1397	0	GLN	A	191	15.5	08 63.	054 7	9.429	1.00	16.52
MOTA	1398	CB	GLN	A	191	12.1	13 62.	829 7	9,721		15.28
ATOM	1399	CG	GLN	A	191	12.5			8.632		17.26
ATOM	1400	CD	GLN	A	191	12.8			9.206		16.84
ATOM	1401	OE1	GLN	A	191	12.0			9.946		21.21
MOTA	1402	NE2	GLN	A	191	14.0			8.864		16.69
MOTA	1403	N	SER	A	192	14.4			9.389		17.04
ATOM	1404	CA	SER			15.5			8.666		15.81

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ATOM	1405	C	SER	A	192	16.899	65.683	79.339	1.00	17.61
MOTA	1406	0	SER	A	192	17.937	65.640	78.700	1.00	16.65
ATOM	1407	CB	SER	A	192	15.209	67.005	78.211	1.00	15.87
MOTA	1408	OG	SER	A	192	14.862	67.833	79.313	1.00	21.12
ATOM	1409	N	ASP	A	193	16.886	65.712	80.681	1.00	17.44
ATOM	1410	CA	ASP	A	193	18.190	65.648	81.360	1.00	15.23
ATOM	1411	C			193	18.889	64.316	81.197	1.00	12.26
ATOM	1412	0			193	20.113	64.204	81.054	1.00	14.16
ATOM	1413	CB			193	18.036	65.832	82.887	1.00	16.01
ATOM	1414	CG			193	17.367	67.116	83.311		14.57
ATOM	1415		ASP			17.503	68.180	B2.691		15.89
ATOM	1416		ASP			16.673	67.038	84.312		19.14
ATOM	1417	N			194	18.025	63.283	81.216		11.48
ATOM	1418	CA			194	18.425	61.882	81.108		13.18
ATOM	1419	С			194	18.851	61.478	79.663		15.63
ATOM	1420	0			194	19.852	60.822	79.387		15.79
ATOM	1421	CB			194	17.210	61.135	81.625	1.00	
ATOM	1422		VAL			17.152	61.202	83.184		19.26
ATOM	1423	CG2				17.079	59.712	81.106		17.70
ATOM	1424	N	ASP			18.050	61.992	78.728		16.54
atom atom	1425 1426	CA	ASP			18.488	61.921	77.332		16.55
ATOM	1427	C	ASP			19.801	62.636	77.029		14.74
ATOM	1428	O CB	ASP			20.758	62.076	76.519		18.92
ATOM	1429	CG	asp asp			17.367 16.139	62.455	76.477		15.91
ATOM	1430	OD1					61.563	76.560		19.86
ATOM	1431	OD2				16.153	60.385	76.922		27.62
ATOM	1432	N N	ALA			15.090	62.069	76.264		26.20
ATOM	1433	CA	ALA			19.902 21.203	63.900	77.450		15.85
ATOM	1434	C	ALA			22.383	64.555	77.312		14.73
ATOM	1435	ō	ALA			23.512	63.806 63.751	77.932 77.429		18.85
ATOM	1436	СВ	ALA			21.134	65.950	77.904		21.06
ATOM	1437	N	ALA			22.056	63.177	79.091		13.59 19.14
ATOM	1438	CA	ALA			23.098	62.442	79.808		17.62
ATOM	1439	C	ALA			23.644	61.202	79.090		17.71
ATOM	1440	ō	ALA			24.851	60.931	79.104		18.18
MOTA	1441	CB	ALA			22.587	62.002	81.181	1.00	
ATOM	1442	N	ARG			22.711	60.477	78.418	1.00	
ATOM	1443	CA	ARG			23.238	59.409	77.565	1.00	
ATOM	1444	C	ARG			24.179	59.843	76.413	1.00	
ATOM	1445	0	ARG	A	198	25.194	59.219	76.113	1.00	
ATOM	1446	СВ	ARG	A	198	22.136	58.469	77.080	1.00	
ATOM	1447	CG	ARG			21.195	58.043	78.179	1.00	
ATOM	1448	ÇD	ARG	A	198	20.142	57.044	77.730		19.20
MOTA	1449	NE	ARG	A	198	19.280	56.629	78.849	1.00	
MOTA	1450	CZ	ARG	A	198	18.003	57.012	79.061	1.00	
ATOM	1451	NH1	ARG	A	198	17.412	57.905	78.325	1.00	
ATOM	1452	NH2	ARG	A	198	17.292	56.518	80,045	1.00	
ATOM	1453	N	VAL			23.907	61.030	75.842	1.00	
ATOM	1454	CA	VAL			24.961	61.510	74.913	1.00	
ATOM	1455	С	VAL			26.376	61.736	75.457	1.00	
ATOM	1456	0	VAL			27.360	61.145	74.988	1.00	
ATOM	1457	CB	VAL			24.452	62.820	74.284	1.00	
ATOM	1458	CG1	VAL .	A	199	25.350	63.540	73.279	1.00	

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MOTA	1459	CG2	VAL	A	199	23.072	62.640	73.680	1.00	15.33
ATOM	1460	N	VAL	A	200	26.471	62.548	76.550	1.00	18.52
ATOM	1461	ÇA	VAL	A	200	27.822	62.723	77.153	1.00	16.27
ATOM	1462	C	VAL	A	200	28.442	61.461	77.794	1.00	14.37
ATOM	1463	0	VAL	A	200	29.643	61.247	77.797	1.00	17.25
MOTA	1464	СВ	VAL	A	200	27.811	63.938	78.100		16.56
ATOM	1465	CG1	VAL	A	200	26.761	63.894	79.246		13.52
ATOM	1466	CG2	VAL	A	200	27.666	65.177	77.226	1.00	17.14
MOTA	1467	N	GLY	A	201	27.556	60.570	78.283		14.98
MOTA	1468	CA	GLY	A	201	27.998	59.297	78.836		13.20
ATOM	1469	С	GLY	A	201	28.609	58.377	77.824	1.00	16.81
ATOM	1470	0	GLY	A	201	29.588	57.701	78.067	1.00	17.03
ATOM	1471	N	SER	A	202	28.034	58.413	76.614	1.00	17.82
ATOM	1472	CA	SER	A	202	28.757	57.750	75.509		16.70
ATOM	1473	C	SER	A	202	30.087	58.390	75.104		13.91
MOTA	1474	0	SER	A	202	31,117	57.765	74.939		18.47
ATOM	1475	CB	SER	A	202	27.813	57.511	74.303		15.94
ATOM	1476	OG	SER	A	202	27.634	58.737	73.600		19.26
ATOM	1477	N	ALA	A	203	30.094	59.719	75.030		14.76
MOTA	1478	CA	ALA	A	203	31.333	60.383	74.641	1.00	14.41
ATOM	1479	С	ALA	A	203	32.527	60.110	75.563		17.92
ATOM	1480	0	ALA	A	203	33.652	59.803	75.177		16.36
MOTA	1481	CB	ALA	A	203	31.042	61.872	74.572		13.61
ATOM	1482	N	VAL	A	204	32.200	60.126	76.880		18.27
MOTA	1483	CA	VAL	A	204	33.290	59.835	77.823		16.45
ATOM	1484	C	VAL	A	204	33.834	58.394	77.718		13.88
ATOM	1485	0	VAL	A	204	35.015	58.177	77.916		17.64
ATOM	1486	CB	VAL	A	204	32.893	60.245	79.285		16.63
ATOM	1487	CG1	VAL	A	204	34.131	60.323	80.177	1.00	16.51
MOTA	1488	CG2	VAL	A	204	31.836	59.301	79.862		13.53
ATOM	1489	N	LAV	A	205	32.971	57.392	77.355		15.31
ATOM	1490	CA	VAL	A	205	33,648	56.100	77.152		17.30
ATOM	1491	С	VAL	A	205	34.636	56,018	75.953		19.20
ATOM	1492	0	VAL			35.644	55.311	76.002		19.08
ATOM	1493	CB	VAL	A	205	32.563	55.014	77.035		19.10
ATOM	1494	CG1	VAL	A	205	32.812	53.549	76.681		16.51
ATOM	1495	CG2	VAL	A	205	31.797	55.088	78.343		17.27
ATOM	1496	N	ALA	A	206	34.409	56.893	74.934		21.03
ATOM	1497	CA	ALA	A	206	35.452	57.018	73.888		19.17
ATOM	1498	С	ALA	A	206	36.765	57.498	74.469		19.27
ATOM	1499	0	ALA	A	206	37.809	56.868	74.353		18.59
MOTA	1500	CB	ALA	A	206	34.982	57.988	72.809		16.60
ATOM	1501	N	THR	A	207	36.643	58.597	75.234		20.20
ATOM	1502	CA	THR	A	207	37.873	59.078	75.903		20.52
ATOM	1503	С	THR	A	207	38.613	58.126	76.838		21.84
ATOM	1504	O	THR	A	207	39.831	58.030	76.898		23.45
ATOM	1505	CB	THR .	A	207	37.659	60.341	76.674		19.84
ATOM	1506	OG1	THR .	A :	207	36.577	61.100	76.137		20.11
ATOM	1507	CG2	THR .	A	207	38.945	61.141	76.710		21.15
MOTA	1508	N	LEU .			37.806	57.345	77.574	1.00	
MOTA	1509	CA	LEU .	A :	208	38.323	56.332	78.510		22.19
ATOM	1510	Ċ	LEU .			39.165	55.285	77.783		25.28
ATOM	1511	0	LEU .			40.232	54.880	78.228		24.41
ATOM	1512	СВ	LEU .			37.170	55.700	79.312		21.93

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ATOM	1513	ÇĢ	LEU	J A	208	36.238	56.370	80.325	1.00 24.99
ATOM	1514	CD1	LEU	J A	208	35.117	55.631	81.058	1.00 26.55
ATOM	1515	CD2			208	37.303	56.685	81.377	1.00 22.67
MOTA	1516	N			209	38.666	54.873	76.588	1.00 24.64
ATOM	1517	CA			209	39.495	53.970	75.766	1.00 20.81
ATOM	1518	С	HIS	A	209	40.863	54.530	75.277	1.00 19.15
ATOM	1519	0	HIS	A	209	41.807	53.872	74.934	1.00 20.30
ATOM	1520	CB	HIS	A	209	38.656	53.449	74.595	1.00 16.87
ATOM	1521	CG	HIS	A	209	37.588	52.476	74.994	1.00 13.42
ATOM	1522		HIS			36.335	52.782	75.375	1.00 14.12
MOTA	1523	CD2	HIS	A	209	37.686	51.099	74.975	1.00 12.37
MOTA	1524	CE1			209	35.653	51.616	75.586	1.00 10.36
ATOM	1525	NE2	HIS	Α	209	36.493	50.590	75.334	1.00 13.53
ATOM	1526	N			21.0	41.035	55.827	75.336	1.00 20.82
ATOM	1527	CA	THR	A	210	42.393	56.384	75.116	1.00 21.40
ATOM	1528	С	THR	A	210	43.396	56.387	76.298	1.00 26.34
MOTA	1529	0	THR	Α	210	44.567	56.745	76.188	1.00 27.83
MOTA	1530	CB	THR	. A	210	42.315	57.836	74.662	1.00 22.02
ATOM	1531	OG1	THR	A	210	42.096	58.723	75.795	1.00 25.04
ATOM	1532	CG2	THR	Α	210	41.307	58.070	73.528	1.00 20.46
ATOM	1533	N	ASN	A	211	42.844	56.032	77.482	1.00 26.32
ATOM	1534	CA	asn	A	211	43.544	56.135	78.782	1.00 23.64
ATOM	1535	С	asn	A	211	44.212	54.824	79.204	1.00 20.62
ATOM	1536	0	ASN	A	211	43.591	53.785	79.374	1.00 22.28
MOTA	1537	CB	ASN	A	211	42.563	56.749	79.827	1.00 24.99
MOTA	1538	CG	ASN	A	211	43.226	56.863	81.206	1.00 24.09
ATOM	1539	OD1	ASN	A	211	43.320	55.886	81.935	1.00 25.25
ATOM	1540	ND2	ASN	A	211	43.689	58.038	81.566	1.00 21.24
MOTA	1541	N	PRO	A	212	45.563	54.884	79.353	1.00 22.01
ATOM	1542	CA	PRO	A	212	46.337	53.660	79.633	1.00 21.52
ATOM	1543	C	PRO	A	212	45.859	52.883	80.848	1.00 22.52
MOTA	1544	0	PRO	A	212	45.670	51.673	80.882	1.00 22.12
ATOM	1545	CB	PRO	A	212	47.743	54.190	79.845	1.00 22.52
ATOM	1546	CG	PRO	A	212	47.805	55.535	79.117	1.00 25.40
ATOM	1547	CD			212	46.391	56.076	79.175	1.00 22.01
ATOM	1548	N			213	45.626	53.674	81.897	1.00 23.96
ATOM	1549	CA			213	45.139	53 .025	83.140	1.00 23.57
ATOM	1550	C	ALA			43.797	52.337	83.019	1.00 21.76
ATOM	1551	٥			213	43.600	51.185	83.403	1.00 24.96
ATOM	1552	СВ	ALA			45.039	54.071	84.259	1.00 21.55
ATOM	1553	N	PHE			42.885	53.085	82.373	1.00 19.30
ATOM	1554	CA	PHE			41.617	52.431	82.017	1.00 20.02
ATOM	1555	C	PHE			41.798	51.170	81,197	1.00 20.38
ATOM	1556	0	PHE			41.255	50.120	81.510	1.00 18.82
ATOM	1557	CB	PHE			40.690	53.445	81.314	1.00 23.58
ATOM	1558	CG	PHE			39.367	52.839	80.840	1.00 26.35
ATOM	1559		PHE			38.249	52.765	81.711	1.00 24.66
ATOM	1560		PHE			39.262	52.365	79.507	1.00 23.08
ATOM	1561		PHE			37.032	52.191	81.265	1.00 27.05
ATOM	1562		PHE			38.052	51.792	79.077	1.00 19.74
ATOM	1563	CZ	PHE			36.951	51.697	79.944	1.00 22.04
ATOM	1564	N ~-	GLN			42.654	51.298	80.149	1.00 22.02
ATOM	1565	CA	GLN			42.904	50.137	79.275	1.00 21.33
MOTA	1566	С	GLN	A	215	43.354	48.889	79.995	1.00 21.73

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ATOM	1567	0	GLN	A	215	42.823	47.783	79.875	1.00	21.17
ATOM	1568	CB	GLN	A	215	43.970	50.520	78.278	1.00	22.37
ATOM	1569	CG	GLN	A	215	43.483	51.517	77.261	1.00	22.30
ATOM	1570	CD	GLN	A	215	44.662	52.026	76.479	1.00	28.18
ATOM	1571	OE1	GLN	A	215	45.821	51.695	76.664	1.00	32.27
ATOM	1572	NE2	GLN	A	215	44.359	52.947	75.605	1.00	28.35
ATOM	1573	N	GLN	A	216	44.375	49.154	80.845	1.00	24.60
MOTA	1574	CA	GLN	A	216	44.876	48.089	81.718	1.00	25.81
MOTA	1575	С	GLN	A	216	43.909	47.530	82.715	1.00	23.07
ATOM	1576	0	GLN	A	216	43.822	46.328	82.899	1.00	22.91
MOTA	1577	CB	GLN	A	216	46.052	48.544	82.517		33.78
ATOM	1578	CG	GLN	A	216	47.181	49.037	81.631	1.00	49,94
ATOM	1579	CD	GLN	A	216	48.161	49.693	82.574	1.00	61.57
ATOM	1580	OE1	GLN	A	216	48.354	49.243	83.704	1.00	69.34
ATOM	1581	NE2	GLN	A	216	48.737	50.805	82.114		63.50
MOTA	1582	N	GLN	A	217	43.155	48.439	83.377	1.00	22.58
MOTA	1583	CA	GLN	A	217	42.099	47.917	84.261		23.69
MOTA	1584	C	GLN	A	217	40.971	47.113	83.590	1.00	24.92
ATOM	1585	0	GLN	A	217	40.480	46.102	84.088	1.00	24.09
ATOM	1586	CB	GLN	Ą	217	41.565	49.042	85.189	1.00	23.44
ATOM	1587	CG	GLN	A	217	40.720	48.541	86.407		23.24
ATOM	1588	CD	GLN			41.489	47.589	87.335		21.58
ATOM	1589	OE1	GLN	A	217	42.676	47.749	87.598		24.82
ATOM	1590	NE2	GLN			40.827	46.516	87.744		19.85
ATOM	1591	N	LEU			40.628	47.595	82.390		26.00
ATOM	1592	CA	LEU			39.701	46.859	81.532		23.47
ATOM	1593	C	LEU			40,195	45.495	81.093		21.96
ATOM	1594	0	LEU			39.476	44.515	81.209		22.51
ATOM	1595	CB	LEU			39.309	47.724	80.327		23.65
MOTA	1596	CG	LEU			38.292	47,073	79.369		21.04
ATOM	1597	CD1	LEU			38.103	47.980	78.168		25,30
ATOM	1598	CD2				36,952	46.736	80.004		13.22
MOTA	1599	N	GLN			41.451	45.425	80.640		23,49
ATOM	1600	CA	GLN			42.033	44.079	80.457		29.37
ATOM	1601	C	GLN			41.880	43.156	81.681		29.60
ATOM	1602	ō	GLN			41.455	42.016	81.569		29.63
ATOM	1603	CB	GLN			43.544	44.131	80.199		37.46
ATOM	1604	CG	GLN			44.052	44.703	78.867		51.74
ATOM	1605	CD	GLN			45.511	45.267	78.911		60.29
ATOM	1606	OE1	GLN			46.415	44.774	79.568		65.45
ATOM	1607	NE2	GLN			45.764	46.352	78.161		60.81
ATOM	1608	N	LYS			42.206	43.722	82.879		28.28
ATOM	1609	CA	LYS			42.004	42.926	84.111		26.68
ATOM	1610	C	LYS			40.588	42.446	84.386		24.54
ATOM	1611	ō	LYS			40.347	41.275	84.640		26.87
ATOM	1612	СВ	LYS			42.591	43.631	85.319		29.93
ATOM	1613			_						
ATOM	1614	CG CD	LYS			44.019 45.015	43.952 44.044	84.934		36.96
ATOM	1615	CE	LYS			44.741	45.121	86.081		47.54
ATOM	1616	NZ	LYS					87.121		55.28
ATOM	1617	N	ALA			44.868 39.630	46.456	86.510		61.92
ATOM	1618	CA	ALA				43.379	84.217		21.04
ATOM	1619	CA	ALA			38.215	42.960	84.307		18.69
ATOM	1620					37.761	41.903	83.291		24.31
ALOM	TOZU	0	ALA	A	221	37.095	40.921	83.598	T.00	26.78

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ATOM			3 ALA A 221	37.306	44.177	84.140	1.00 14.85
MOTA			LYS A 222	38.223	42.106		
ATOM			LYS A 222	38.065			
ATOM		-	LYS A 222	38.668	39.675		
ATOM			LYS A 222	38.023			
MOTA		S CB		38.591			
ATOM	1627	7 CG	LYS A 222	37.682			
ATOM		CD	LYS A 222	38.038			
ATOM	1629	CE	LYS A 222	37.050		77.109	1.00 22.07
ATOM	1630	NZ	LYS A 222	37.556			
ATOM	1631		ALA A 223	39,949			
ATOM	1632		ALA A 223	40.533			1.00 25.17
MOTA	1633		ALA A 223	39.812			1.00 26.19
ATOM	1634	0	ALA A 223	39.534	36.652	83.573	1.00 27.99
ATOM	1635		ALA A 223	42.013	38.638	82.691	1.00 21.36
ATOM	1636	N	GLU A 224	39.424	38.696	84.487	1.00 27.72
ATOM	1637		GLU A 224	38.643	38.187	85.610	1.00 27.59
ATOM	1638		GLU A 224	37.338	37.525	85.191	1.00 28.89
ATOM	1639		GLU A 224	36.971	36.412	85.568	1.00 28.75
ATOM	1640	CB	GLU A 224	38.476	39.348	86.606	1.00 29.18
ATOM	1641		GLU A 224	37.470	39.099	87.741	1.00 29.05
ATOM	1642		GLU A 224	37.335	40.348	88.557	1.00 29.41
ATOM	1643	OE1	GLU A 224	36.506	41.189	88.269	1.00 28.08
ATOM	164,4	OE2	2 GLU A 224	38.060	40.487	89.516	1.00 31.14
ATOM	1645	N	PHE A 225	36.659	38.233	84.288	1.00 29.33
ATOM	1646	CA	PHE A 225	35.398	37.699	83,770	1.00 28.70
ATOM	1647	С	PHE A 225	35.551	36.354	83.061	1.00 31.53
ATOM	1648	0	PHE A 225	34.802	35.399	83.222	1.00 29.17
ATOM	1649	CB	PHE A 225	34.786	38.756	82.858	1.00 26.48
ATOM	1650	CG	PHE A 225	33.449	38.281	82.361	1.00 25.90
ATOM	1651		PHE A 225	32.361	38.258	83.250	1.00 28.71
ATOM	1652	CD2	PHE A 225	33.317	37.854	81.022	1.00 28.64
ATOM	1653		PHE A 225	31.129	37.758	82.817	1.00 29.98
MOTA	1654		PHE A 225	32.074	37.365	80.564	1.00 28.53
ATOM	1655	CZ	PHE A 225	30.998	37.309	81.479	1.00 30.02
MOTA	1656	N	ALA A 226	36.635	36.308	82.289	1.00 31.91
ATOM	1657	CA	ALA A 226	36.996	35.055	81.643	1.00 36.17
ATOM	1658	С	ALA A 226	37.178	33.829	82.536	1.00 40.94
ATOM	1659	0	ALA A 226	36.704	32.735	82.271	1.00 41.75
ATOM	1660	CB	ALA A 226	38.284	35.261	80.877	1.00 33.81
MOTA	1661	N	GLN A 227	37.883	34.081	83.647	1.00 44.91
ATOM	1662	CA	GLN A 227	38.067	32.931	84.543	1.00 49.32
ATOM	1663	C	GLN A 227	36.782	32.579	85.233	1.00 51.62
ATOM	1664	0	GLN A 227	36.396	31.467	85.528	1.00 50.02
ATOM	1665	СВ	GLN A 227	39.239	33.150	85.499	1.00 50.80
ATOM	1666	CG	GLN A 227	40.441	33.937	84.900	1.00 59.92
ATOM	1667	CD	GLN A 227	40.812	33.681	83.397	1.00 68.68
ATOM	1668	OE1	GLN A 227	40.799	32.592	82.834	1.00 73.97
ATOM	1669		GLN A 227	41.221	34.764	82.726	1.00 65.71
ATOM	1670	N	HIS A 228	36.041	33.669	85.411	1.00 58.91
ATOM	1671	CA	HIS A 228	34.687	33.501		1.00 65.53
ATOM	1672	C	HIS A 228	33.816	32.519		1.00 68.04
ATOM	1673		HIS A 228		31.764		1.00 67.46
ATOM	1674	CB	HIS A 228	34.091	34.903		1.00 68.11

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ATOM	1675	CG	BIC	D	228	32.632	34.780	86.283	1.00 71.87
ATOM	1676		HIS			31.729	34.968	85.313	1.00 74.51
ATOM	1677								
			HIS			32.007	34.363	87.458	1.00 75.53
ATOM	1678		HIS			30.511	34.660	85.852	1.00 78.68
ATOM	1679		HIS			30.682	34.288	87.171	1.00 80.02
MOTA	1680	N			229	34.061	32.545	83.846	1.00 74.45
ATOM	1681	CA	GLN	A	229	33.306	31.659	82.963	1.00 81.06
MOTA	1682	С	GLN	A	229	33.569	30.149	83.028	1.00 85.59
ATOM	1683	0	GLN	A	229	33.123	29.427	82.135	1.00 86.52
MOTA	1684	CB	GLN	A	229	33.477	32.181	81.530	1.00 81.47
ATOM	1685	CG	GLN	A	229	33.002	33.631	81.335	1.00 81.55
ATOM	1686	CD	GLN	A	229	31.488	33.690	81.352	1.00 83.81
ATOM	1687	OE1			229	30.804	33.832	82,355	1.00 82.35
ATOM	1688	NE2			229	30.950	33.588	80.141	1.00 86.80
ATOM	1689	N			230	34.317	29.749	84.086	1.00 91.08
ATOM	1690	CA			230	34.965	28.444	84.325	1.00 95.45
	1691				230		27.245		
ATOM		CB				33.976		84.052	1.00 97.51
ATOM	1692	CG			230	34.256	26.053	83.073	1.00 98.30
ATOM	1693	CD			230	34.035	26.121	81.534	1.00 98.30
ATOM	1694	CE			230	34.810	27.172	80.713	1.00100.00
ATOM	1695	NZ			230	36.244	27.239	81.067	1.00100.00
MOTA	1696	C			230	36.409	28.279	83.743	1.00 97.06
ATOM	1697	OCT1	LYS	A	230	36.876	29.108	82.942	1.00 95.86
ATOM	1698	OCT2	LYS	A	230	37.052	27.241	83.957	1.00 99.89
ATOM	1935	S	SO4	S	231	22.561	63.872	89.148	1.00 45.29
ATOM	1936	01	504	s	231	21.748	62.858	88.279	1.00 50.45
ATOM	1937	02	SO4	S	231	21.648	64.707	90.036	1.00 51.74
ATOM	1938	03			231	23.551	63.095	90.035	1.00 49.75
ATOM	1939	04			231	23.260	64.912	88.285	1.00 44.08
ATOM	1	0			232	10.522	63.513	85.670	1.00 17.86
ATOM	2	Ö			233	34.116	63.633	80.578	1.00 20.45
	3	Ö					61.775		
MOTA					234	7.928		88.229	1.00 15.62
ATOM	4	0			235	10.374	64.545	82.597	1.00 14.58
ATOM	5	0			236	15.375	75.641	85.508	1.00 22.07
ATOM	6	0			237	20.773	44.507	86.785	1.00 18.67
MOTA	7	0	нон	M	238	32.701	49.912	75.935	1.00 15.79
MOTA	8	0	нон	M	239	21.979	72.096	84.493	1.00 19.08
ATOM	9	0	нон	W	240	13.158	73.905	82.705	1.00 27.34
ATOM	10	0	HOH	W	241	14.358	71.880	73.410	1.00 26.83
ATOM	11	0	нон	W	242	5.537	80.043	74.802	1.00 23.33
ATOM	12	0	нон	W	243	36.136	62.604	78.407	1.00 23.19
ATOM	13	0	нон			30.393	53.028	87.579	1.00 19.02
ATOM	14	0	нон			28.532	49.107	93.252	1.00 21.32
ATOM	15	ō	нон			24.657	73.146	75.882	1,00 20.92
ATOM	16	ō	нон			10.080	55.567	81.848	1.00 33.80
ATOM	17	o	нон			29.907	52.840	73.379	1.00 33.00
		_							
ATOM	18	0	нон			38.583	48.054	74.575	1.00 24.10
MOTA	19	0	нон			29.465	68.020	86.676	1.00 32.30
ATOM	20	0	нон			12.847	73.680	85.460	1.00 40.76
ATOM	21	0	нон			5.516	59.770	95.129	1.00 40.84
ATOM	22	0	HOH			42.504	47.354	77.319	1.00 30.77
ATOM	23	O	нон			13.495	75.378	74.412	1.00 22.57
ATOM	24	0	HOH	M	255	17.100	76.564	77.737	1.00 30.00
ATOM	25	0	HOH	W	256	33.508	40.103	102.712	1.00 26.49

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ATOM	26	O	HOH W	257	20.825	55.64	8 81,278	1.00	20.11
ATOM	27	0	HOH W	258	19.730	61.70	1 89.970	1.00	23.10
ATOM	28	0	HOH W		4.363			1.00	33.74
ATOM	29	0	HOH W	260	31.490	42.65	98.480	1.00	34.19
ATOM	30	0	нон w		6.696	75.130	78.477	1.00	15.66
ATOM	31	0	HOH W		10.667	67.023	3 75.103	1.00	38.86
ATOM	32	0	HOH W		8.252	64.433	92.307	1.00	23.15
ATOM	33	0	HOH W		41.924	51.223	3 74.247	1.00	30.19
ATOM	34	0	HOH W		1.437	67.705	89.398	1.00	39.48
ATOM	35	0	HOH W		4.055	66.946	91.467	1.00	29.22
ATOM	36	0	нон и 2		3.092	69.112		1.00	25.58
ATOM	37	0	нон w		9.537	59.065			30.90
ATOM	38		HOH W		9.306	83.197		1.00	44.19
ATOM	39	0	HOH W 2		34.786	41.166			32.98
ATOM	40	0	HOH W 2		28.084	37.193			30.43
ATOM	41	0	нон w		40.742	49.227			21.82
ATOM ATOM	42	0	HOH W 2		35.074	40.712			29.87
ATOM	43	0	HOH W 2		30.318	45.526			35.57
ATOM	44 45	0	HOH W 2		31.493	69.162			19.51
ATOM	46	0	HOH W 2		42.914	61.700			28.69
ATOM	47	o	HOH W 2		34.422	64.714			38.81
ATOM	48	Ö	HOH W 2		13.405	78.374			25.22
ATOM	49	ő	HOH W 2		44.634	57.811			31.73
ATOM	50	o	HOH W 2		44.303 32.596	60.992			28.14
ATOM	51	ő	HOH W 2		22.182	51.432			22.63
ATOM	52	ŏ	HOH W 2		18.482	40.126			27.50
ATOM	53	ō	HOH W 2		36.960	55.362			21.25
ATOM	54	ō	HOH W 2		35.881	42.360 48.845	74.192 94.047		28.88
ATOM	55	ō	HOH W 2		26,212	59.698			26.90
ATOM	56	ō	HOH W 2	-	29.246	44.303	94.760 73.369		23.37
ATOM	57	o	HOH W 2		27.356	35.947	80.422		40.38
ATOM	58	ō	HOH W 2		40.482	45.029	76.766		31.74
ATOM	59	ō	HOH W 2		24.864	58.724	91.112		30.88 25.30
ATOM	60	0	нон w 2		28.560	61.547	91.755		39.37
ATOM	61	0	нон w 2		27.888	63.113	90.252		40.28
ATOM	62	0	нон w 2		31.069		103.435		38.13
ATOM	63	0	нон w 2		5.144	47.860	86.978		37.63
ATOM	64	O	HOH W 2		29.373	52.425	90.409		21.69
ATOM	65	0	HOH W 2	96	41.571	51.401	87.864		31.72
ATOM	66	0	HOH W 2	97	35.633		101.396		42.27
ATOM	67	0	HOH W 2	98	35.257	40.157	78.063		30.17
ATOM	68	0	HOH W 2	99	33.734	71.189	79.910	1.00	
ATOM	69	0	HOH W 3	00	17.659	69.593	75.158	1.00	
ATOM	70	0	нон w з	01	17.005	72.932	72,774		33.93
ATOM	71	0	нон w з	02	15.769	48.059	85.107	1.00	
ATOM	72	0	HOH W 3	03	15.023	64.697	75.333	1.00	
ATOM	73	0	HOH W 3		13.546	67.305	74.469	1.00	
MOTA	74	0	нон и з	05	30.044	75.863	82.738	1.00	
ATOM	75	0	HOH W 30		5.253	66.383	98.323	1.00	
ATOM	76	0	нон м з		25.914	72.829	89.073	1.00	
ATOM	77	0	нон w з		38.474	67.620	76.050	1.00	
ATOM	78	0	HOH W 30		34.101	41.534	100.215	1.00	
ATOM	79	0	нон и за	10	29.974	37.419	76.650	1.00	
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【図43】

ATOM	80	0	HOH W	311	17.829	44.406	81.773	1.00 29.29
ATOM	81	0	HOH W	312	17.766	66.478	75.705	1.00 34.51
ATOM	82	0	HOH W	313	35.983	70.225	78.152	1.00 38.85
ATOM	83	0	HOH W	314	18.063	58.644	75.592	1.00 33.24
ATOM	84	0	HOH W	315	17.740	45.824	75.692	1.00 31.29
ATOM	85	0	HOH W	316	21.442	55.668	101.498	1.00 30.06
ATOM	86	0	HOH W	317	30.660	37.639	105.501	1.00 46.34
ATOM	87	0	нон w	318	28.143	47.582	99.410	1.00 71.00
ATOM	88	0	HOH W	319	11.398	65.394	76.821	1.00 34.86
MOTA	89	0	HOH W	320	31.737	45.760	98.744	1.00 38.11
ATOM	90	0	HOH W	321	16.084	45.559	87.137	1.00 43.68
MOTA	91	0	BOH M	322	36.498	37.962	78.989	1.00 35.45
ATOM	92	0	HOH W	323	41.868	42.172	76.980	1.00 56.04
MOTA	93	0	HOH W	324	44.704	68.004	76.606	1.00 73.28
ATOM	94	0	HOH W	325	30.214	44.935	101.119	1.00 28.63
ATOM	95	0	HOH W	326	43.719	69.244	83.004	1.00 32.20
ATOM	96	0	HOH W	327	7.992	54.768	93.490	1.00 36.05
ATOM	97	0	HOH W	328	11.059	49.604	75.476	1.00 43.80
MOTA	98	0	HOH W	329	17.730	37.202	79.516	1.00 44.41
ATOM	99	0	нон w	330	14.170	59.796	74.913	1.00 70.26
ATOM	100	Q	HOH W	331	28.648	70.326	88.645	1.00 34.35
ATOM	101	O	HOH W	332	16.146	57.197	73.492	1.00 49.27
ATOM	102	0	HOH W	333	11.086	52.502	82.116	1.00 39.47
ATOM	103	0	HOH W	334	15.950	60.744	73.392	1.00 63.16
ATOM	104	0	HOH W	335	23.809	74.443	89.142	1.00 63.73
ATOM	105	a	HOH W		43.077	70.945	86.543	1.00 41.77
ATOM	106	0	HOH W		44.625	68.578	85.466	1.00 42.53
ATOM	107	0	HOH W	338	38.003	70.941	79.707	1.00 47.97
ATOM	108	O	HOH W		42.635	39.826	86.317	1.00 39.90
ATOM	109	0	HOH W		28.158	51.028	97.893	1.00 35.28
ATOM	110	0	HOH W	341	34.562	57.666	98.193	1.00 56.42
ATOM	111	0	HOH W	342	23.659	34.535	79.197	1.00 84.39
MOTA	112	0	HOH W		10.337	58.458	76.704	1.00 45.85
ATOM	113	0	HOH W	344	32.164	75.101	85.461	1.00 54.21
MOTA	114	0	HOH W	345	32.930	38.410	86.586	1.00 43.15
ATOM	115	o	HOH W	346	32.310		102.558	1.00 47.71
MOTA	116	0	HOH W		11.163	49.101	82.634	1.00 84.37
MOTA	117	0	нон и		34.268	69.634	83.019	1.00 47.39
MOTA	118	0	нон ш		31.352	37.085	89.579	1.00 74.88
ATOM	119	0	HOH W		29.118	56.986	95.860	1.00 34.59
ATOM	120	0	HOH W		1.634	70.786	81.659	1.00 41.89
ATOM	121	0	HOH W		2.044	71.714	85.736	1.00 37.84
MOTA	122	0	HOH W		16.219	75.511	74.471	1.00 44.53
ATOM	123	0	HOH W		24.035	45.705	97.204	1.00 48.11
ATOM	124	0	HOH W		17.939	77.382	82.853	1.00 65.65
MOTA	125	o	HOH W		12.504	76.991	70,634	1.00 50.43
MOTA	126	0	HOH W		16.951	78.295	74.889	1.00 47.02
MOTA	127	ō	HOH W		15.777	75.404	81.566	1.00 33.68
ATOM	128	o	нон м		37.401	72.376	82.831	1.00 50.52
ATOM	129	ō	нон м		14.060	44.359	88.918	1.00 80.84
ATOM	130	ō	HOH W		32.619	76.123	75.757	1.00 42.84
ATOM	131	ō	HOH W		21.836	66.226	94.339	1.00 63.40
ATOM	132	ō	HOH W		16.011	46.526	82.837	1.00 38.42
ATOM	133	ō	HOH W		7.716	57.886	82.470	1.00 50.22
		_			7.710	27.000	U 1 / U	1.00 30.22

【図44】

ATOM	134	0	HOH W	365	41.813	72.155	81.960	1.00	76.60
ATOM	135	0		366	5.810	63.614	94.440	1.00	41.72
ATOM	136	0	HOH M	367	22.833	66.006	98.308	1.00	65.79
ATOM	137	0		368	21.384	36.791	76.692		59.29
ATOM	138	0	HOH W	369	38.765	52.950	92.219		27.87
MOTA	139	0	HOH W		46.430	68.991	81.609		70.52
ATOM	140	0	HOH W	371	36.973	69.709	83.153	1.00	33.81
MOTA	141	0	HOH W	372	22.238	43.716	92.825		44.49
ATOM	142	0	HOH W	373	23.096	80.189	77.308		50.73
ATOM	143	0	HOH W	374	2.790	53.932	81.478		50.22
ATOM	144	0	HOH W		3.292	64.768	94.055	1.00	46.64
ATOM	145	0	HOH W		26.937	79.257	75.755		47.21
ATOM	146	0	HOH W		45.046	50.594	85.873		46.10
ATOM	147	0	HOH W		24.988	68.312	90.158		39.03
MOTA	148	0		379	2.045	61.203	93.643		49.73
ATOM	149	0	HOH W		44.273	56.110	87.700		46.74
ATOM	150	0	HOH W		26.747	76.462	73.043	1.00	50.91
MOTA	151	0	HOH W		40.545	70.889	76.918		68.80
ATOM	152	0	HOH W		25.523	80.486	83.807		67.90
MOTA	153	0	HOH W		40.972	36.296	87.372		66.49
ATOM	154	0	HOH W		12.617	56.710	77.567		44.81
ATOM	155	0	HOH W		44.460	48.054	74.082		41.02
ATOM	156	0	HOH W		35.781	73.896	86.117		38.14
ATOM	157	0	HOH W		21.625	80.398	81.815		47.96
ATOM	158	0	HOH W		46.628	56.635	82.977		50.89
ATOM	159	0	HOH W		12.308	51.573	78.083		64.92
ATOM	160	0	HOH W		30.773	39,420	87.798		55.92
ATOM	161	0	нон w		26.088	65.110	89.923		44.80
ATOM	162	0	нон w		10.719	70.886	96.928		48.46
ATOM	163	0	HOH W		12.474	47.243	84.457		53.08
ATOM	164	0	HOH W		24.296	71.312	91.828		48.39
ATOM	165	0	HOH W		6.459	50.108	83.133		57.82
ATOM	166	0	HOH W		42.423	66.213	75.196		34.97
ATOM	167	0	HOH W		29.045		101.769		46.59
ATOM	168	0	HOH W		27.195		105.406		41.59
ATOM	169	0	HOH W		6.834	56.385	96.211		45.81
ATOM	170	0	нон м		47.957	50.138	78.280		43.60
ATOM	171	0	HOH W		23.330	36.461	72.787		54.41
ATOM	172	0	м нон		29.051	79.533	81.900		78.87
ATOM	173	0	W HOH		46.670	55.026	74.340		68.61
ATOM	174	0	HOH W		28.985	78.746	85.840		75.16
ATOM ATOM	175 176	0	HOH W		32.117	68.589	73.365		42.10
ATOM	177	0	HOH W		48.677	52.842	75.727		66.77
ATOM	178	0	HOH W		29.185	36.245	72.017		75.24
ATOM	179	0	HOH W		37.168 11.986	67.596	97.670		38.24
ATOM	180	ō	HOH W		39.548	77.352	92.370		35.94
ATOM	181				30.500	63.174	98.280		39.58
ATOM	182	0	W HOH W		18.003	79.967	79.292		62.87
ATOM	183	0	HOH W		34.455	41.205	83.764		64.48
ATOM	184	0	HOH W			37.242	89.080		52.58
ATOM	185	0	HOH W		47.074 10.880	60.938	83.746		66.98
ATOM	186	0	HOH W			54.535	78.559		45.29
ATOM	187	0			30.230	76.830	74.341		71.09
UTANI	TO!	U	HOH W	-≉TΩ	12.118	81.147	79.341	1.00	49.66

【図45】

ATOM	188	0	HOH W	419	32.095	76.945	80.548	1.00	63.23
ATOM	189	0	HOH W	420	-0.301	68.264	84.539	1.00	48.83
ATOM	190	0	HOH W	421	10.822	64.227	102,313	1.00	81.32
MOTA	191	0	HOH M	422	23.374	42.555	101.170	1.00	42.91
ATOM	192	0	HOH W	423	20.016	59.713	74.793	1.00	38.45
ATOM	193	0	HOH W	424	15.833	78.412	79.495	1.00	56.01
ATOM	194	0	W HOH	425	43.534	35.850	84.957	1.00	63.16
ATOM	195	0	HOH W	426	11.933	68.018	98.874	1.00	52.15
ATOM	196	0	нон w	427	20.777	37.368	85.962	1.00	57.71
ATOM	197	0	HOH W	428	22.392	36.632	89.560	1.00	68.43
ATOM	198	0	нон м	429	29.340	37.487	101.980	1.00	74.20
ATOM	199	0	HOH W	430	23.237	39.294	91.878	1.00	74.07
MOTA	200	0	нон м		13.654	75.325	94.697	1.00	73.83
MOTA	201	0	HOH W		27.904	38.307	96.631		57.14
ATOM	202	0	HOH W		44.213	59.909	79.188		37.71
ATOM	203	0	HOH M		2.129	75.408	79.755		64.17
ATOM	204	0	HOH W		13.993	43.469	84.483		59.78
MOTA	205	0	HOH W		31.644	55.529	99.951		58.81
ATOM	206	0	HOH W		9.462	82.415	76.470		48.44
ATOM	207	0	HOH W		21.813	58.761	98.061		60.37
ATOM	208	0	HOH W		22.202	59.533	93.382		43.39
ATOM	209	0	HOH W		18.118	43.497	86.455		46.86
ATOM	210	0	HOH W		13.762		105.466		57.78
ATOM	211	0	HOH M		33.277	73.931	83.853		56.73
ATOM	212	О	HOH W		34.442	68.648	90.744		27.90
ATOM	213	0	HOH W		30.640	67.899	91.831		53.48
MOTA	214	0	HOH W		40.813	44.217	74.058		53.35
ATOM	215	0	HOH W		33.012	71.334	90.213		53.98
ATOM	216	0	HOH W		25.130	57.928	101.293		38.97
ATOM	217	0	HOH W		7.584	82.067	74.163		26.55
ATOM	218	0	HOH W		42.214	40.521	78.980		37.75
ATOM	219	0	HOH W		8.915		101.115		50.37
ATOM	220	0	HOH W		15.963	42.582	79.699		71.24
ATOM	221	0	HOH W		23.011	77.967	75.363		64.04
ATOM	222	0	HOH W		36.910	35.452	88.469		70.47
MOTA	223	0	HOH W		37.814	55.271	99.966		54.36
ATOM	224	0	HOH W		26.721	58.439	99.230		86.46
MOTA	225	0	HOH W		16.108	40.093	81.126		98.28
ATOM	226	0	HOH W		27.800	35.543	96.536		63.56
MOTA	227	0	HOH W		5.859	51.318	95.801		68.96
ATOM ATOM	228	0	HOH W		7.841	51.875	96.622		64.76
ATOM	229 230	0	HOH W		28.280	66.535	89.122		73.27
ATOM	231	0	HOH W		13.943	46.268	81.680		56.20
ATOM	231	0	HOH W		14.681	69.220	73.344		83.46
ATOM	232	0		464	30.388 8.062	71.379 56.915	89.815 75.809		60.45 59.76
ATOM	233	0		465		41.907			
ATOM	235	0		466	30.104 4.988	41.907	101.688		61.89
ATOM	236	0		467	8.747	53.997	95.471 77.187		61.48
END	230	•	HOR W	10/	0./%/	33.331	11.101	1.00	13.14
131112									

【図46】

A72F(s) 5'-CA-GAC-CTG-GCC-TTT-GGC-GAT-GTG-GC-3'
A72F(as) 3'-GT-CTG-GAC-CGG-AAA-CCG-CTA-CAC-CG-5'

D L A F72 G D V

A72E(s) 5'-CA-GAC-CTG-GCC-GAA-GGC-GAT-GTG-GC-3'

A72E(as) 3'-GT-CTG-GAC-CGG-CTT-CCG-CTA-CAC-CG-5'

D L A E72 G D V

【図47】

I103D(B) 5'-TG-ACC-AAT-ATG-GAC-GAG-GAC-GCC-GG-3'

I103D(as) 3'-AC-TGG-TTA-TAC-CTG-CTC-CTG-CGG-CC-5'

T N M D103 E D A

T153N(s) 5'-GG-CAT-ACC-TCT-AAC-GGC-TGG-GCT-AC-3'

T153N(as) 3'-CC-GTA-TGG-AGA-TTG-CCG-ACC-CGA-TG-5'

H T S N153 G W A

[図48]

L140F(s) 5'-AC-CAG-GAC-AAA-TTC-TCC-AAA-AAT-GG-3'

L140F(as) 3'-TG-GTC-CTG-TTT-AAG-AGG-TTT-TTA-CC-5'

Q D K F140 S K N

L140K(s) 5'-AC-CAG-GAC-AAA-AAA-TCC-AAA-AAT-GG-3'

L140K(as) 3'-TG-GTC-CTG-TTT-TTT-AGG-TTT-TTA-CC-5'

Q D K K140 S K N

L140E(s) 5'-AC-CAG-GAC-AAA-GAA-TCC-AAA-AAT-GG-3'

L140E(as) 3'-TG-GTC-CTG-TTT-CTT-AGG-TTT-TTA-CC-5'

Q D K E140 S K N

【手続補正書】

【提出日】平成12年9月14日(2000.9.14)

【手続補正1】

【補正対象書類名】明細書

【補正対象項目名】0050

【補正方法】変更

【補正内容】

【0050】実施例20 エンテロバクター・アエロゲネス IFO 12010 由来野生型酸性ホスファターゼの精製とN末端アミノ酸配列の決定

特開平10-201481号公報の実施例24記載のエシェリヒア・コリJM109/pENP110の培養菌体からエンテロバクター・アエロゲネスIF012010由来の酸性ホスファターゼを精製してN末端アミノ酸配列を決定し、成熟蛋白質のアミノ酸配列を決定した。エシェリヒア・コリ JM109/pENP1 10はエンテロバクター・アエロゲネスIF012010由来の酸性ホスファターゼ遺伝子をエシェリヒア・コリJM109株に導入した菌で、該酸性ホスファターゼを生産する。該酸性ホスファターゼ遺伝子の塩基配列より予想される前駆体蛋白質のアミノ酸配列は配列表の配列番号10に示すアミノ酸配列は、L610/A630/E64A/N67D/S69A/G72D/T133K/E134D/I151 T変異型EA-APのアミノ酸配列である。ペプトン1g/dl、酵母エキス0.5g/dl及び食塩1g/dlを含有する栄養培地

(pH7.0) 50mlを500ml坂口フラスコに入れ、120℃にて2 0分間加熱殺菌した。これにエシェリヒア・コリ JM109/ pENP110を一白金耳接種し、30℃で16時間振とう培養し た。培養液から遠心分離により菌体を回収した菌体を10 0 mlの100mMリン酸カリウムバッファー(pH7.0) に懸 濁し、4℃で20分間超音波処理を行い菌体を破砕した。 処理液を遠心分離して不溶性画分を除き、無細胞抽出液 を調製した。この無細胞抽出液に30%飽和となるように 硫酸アンモニウムを添加した。遠心分離により生成した 沈殿を除去した後、上清液に60%飽和となるように硫酸 アンモニウムを追加添加した。生成した沈殿を遠心分離 により回収し、100mMリン酸カリウムバッファーに溶解 した。この粗酵素液を100mMリン酸カリウムバッファー (pH7.0) 500mlに対し3回透析した後、20mMリン酸カリ ウムバッファー (pH7.0) で平衡化したDEAE-トヨパール 650Mカラム(φ 3.0×10.0cm)にチャージし、20mMリン 酸カリウムバッファー (pH7.0) で洗浄した。リン酸転 移活性は素通り画分にあったので、当該画分を回収し た。 この活性画分に35%飽和となるように硫酸アンモ ニウムを添加し、これを35%飽和硫酸アンモニウムを含 む20mMリン酸カリウムバッファー (pH7.0) で平衡化し たブチルトヨパールカラム (φ3.0×7.0cm) に吸着させ た。これを35%飽和から20%飽和リン酸カリウムバッフ ァー (pH7.0) の直線的な濃度勾配で溶出した。活性画

分を集め、10mMリン酸カリウムバッファー (pH6.0) 1 Lに対し透析した後、10mMリン酸カリウムバッファー (pH6.0) で平衡化したCM-トヨパール カラム (φ3.0× 7.0cm) に吸着させた。これをOmMから300mM 塩化カリウ ムを含むリン酸カリウムバッファー (pH6.0) の直線的 な濃度勾配で溶出した。この活性画分を集めた。以上の 操作によって、リン酸転移活性を示す酵素を無細胞抽出 液より最終的に約16%の回収率で約5倍に精製した。こ の酵素標品は、SDSーポリアクリルアミド電気泳動に おいて均一であった。本精製酵素をDITC メンブレ ン〔ミリゲン/バイオサーチ(Milligen/Biosearch)社 製〕に吸着させ、Prosequencer 6625(ミリゲン/バイ オサーチ社製)を用いてN末端のアミノ酸配列を決定し たところ配列表の配列番号98に示した5残基のN末端 のアミノ酸配列が決定された。精製酵素のN末端は配列 表の配列番号10の配列の21番目のアラニン残基から開 始していたため、配列表の配列番号10に示されるアミ ノ酸配列は前駆体蛋白質の配列であり、1番目のメチオ ニン残基から20番目のフェニルアラニン残基までのペプ チドは翻訳後に除去されるものと考えられた。この結果 より成熟体蛋白質のアミノ酸配列は配列表の配列番号1 0に示される配列中、アミノ酸番号1~228に示され る配列に相当する。

【手続補正2】

【補正対象書類名】明細書

【補正対象項目名】0062

【補正方法】変更

【補正内容】

【0062】実施例23 グアノシンに対する親和性の向上したエンテロバクター・アエロゲネス IFO 12010 由来新規変異型酸性ホスファターゼ遺伝子導入菌によるグアノシンのリン酸化反応

それぞれの変異型酸性ホスファターゼ遺伝子を含むプラ スミドを導入したエシェリヒア・コリ JM109/pENP18 0、エシェリヒア・コリJM109/pENP320、エシェリヒ ア・コリJM109/pENP340、エシェリヒア・コリJM10 9/pENP410、エシェリヒア・コリJM109/pENP510、及 びエシェリヒア・コリ J M 109/pENP520をアンピシリン 100 µg/ml及び I P T G1mMを含む L 培地50m I に接種 し、37℃で16時間培養した。ピロリン酸10g/dl、及び特 願平12-189226号明細書の実施例1と同様に調製した粉 砕グアノシン6.6g/dlを100mM酢酸バッファー (pH4. 5) に溶解し、これにそれぞれの菌体を乾燥菌体重量で1 00mg/dlとなるように添加し、pHを4.5に維持しなが ら、35℃で12時間反応させた。生成した5′ーグアニル酸 の量を表17に示した。表17に示すように変異型酵素 を導入した菌はいずれも親株であるエシェリヒア・コリ JM109/pENP180に比べて生産性が向上し、高い収率 で5'ーグアニル酸を生成蓄積した。

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